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QUALITY, SIZE, CAPACITY, GROSS ANATOMY, AND HISTOLOGY OF COW UDDERS IN RELATION TO MILK PRODUCTION [1]

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INTRODUCTION

The generally persistent belief among teachers and other professional men in dairy-cattle and veterinary work that the capacity for storing milk in the cow's udder is not more than about one-half pint to each quarter, and that the greater part of the milk obtained at any milking is secreted during the milking process, has been discussed in a previous publication.[3] Evidence has been presented which shows beyond question that a very large proportion, if not all, of the milk obtained at any milking is stored within the udder before the milking process is commenced.[4]

The purpose of this paper is to discuss the quality, size, and general characteristics of the udders used in post-mortem milking studies; to describe and illustrate their gross and microscopic anatomy; to show that not only is the milk stored within the udder, but that the capacity of the secretory system is sufficient to hold considerably more than the quantity produced at a milking; and to show the relation of udder characteristics to the demonstrated producing ability of the cow.

The regular procedure adopted for studying the mammary gland in relation to its producing capacity is to examine it frequently and regularly through its different stages of development and activity, to determine its size (empty weight), to measure the capacity of its secretory system after amputation, and to study the gross and microscopic structure and the physical properties of its tissue.

METHODS OF STUDY

JUDGING THE CHARACTERISTICS OF THE UDDER BY ANTE-MORTEM EXAMINATION

Ante-mortem examinations to determine the comparative type, quality, and general characteristics of udders are made on all cows in the breeding herd of the Bureau of Dairy Industry at Beltsville, Md. Observations of this character have been made on more than 250 cows.

[1] Received for publication Feb. 4, 1932; issued November, 1932.

[2] The histological study of the udders on which this report is based was made possible through the courtesy of J. R. Mohler, Chief of the Bureau of Animal Industry, who made available the personnel and facilities of the Pathological Division for the conduct of that phase of the work.

[3] SWETT, W. W. RELATION OF THE CONFORMATION AND ANATOMY OF THE DAIRY COW TO HER MILK AND BUTTERFAT PRODUCING CAPACITY. UDDER CAPACITY AND MILK SECRETION. Jour. Dairy Sci. 10: 1–14. 1927.

[4] ———. Op. cit.

——— MILLER, F. W., and GRAVES, R. R. QUANTITY OF MILK OBTAINED FROM AMPUTATED COW UDDERS. Jour. Agr. Research 45: 385-400, illus. 1932.

In most cases monthly observations have been made through at least one lactation period. The condition of the udder is determined by visual examination, by manipulation, and by palpation. More than 30 items are recorded at each examination. Only a few of the items which may be significant in relation to "quality of udder" are discussed in this paper. These are (1) looseness of udder, (2) "yieldability" of udder, (3) softness and mellowness of gland tissue, (4) abundance of fiber, and (5) average free space.

The looseness of the udder is the extent to which its covering can be stretched. The "yieldability" of the udder is the degree to which it responds to pressure. The softness and mellowness of the gland tissue is the extent to which it responds to kneading, and is judged entirely by palpation. The abundance of fiber present in the gland tissue also is determined by palpation. Gland tissue which is rough and harsh and appears to be corrugated on the surface is classed as fibrous. That which feels smooth and of uniform consistency is classed as not fibrous. The free space above each teat is the distance above the base of the teat which can readily be compressed without marked resistance. This is determined for each quarter, but since a considerable variation is found in the different quarters of the same udder, the average is used as an index of free space for the udder.

It is recognized that in any grading system based on judgment, the personal element can not be entirely avoided. The use of such a system, however, is necessary in dealing with factors that can not be definitely measured, and is considered satisfactory for this study.

Looseness and yieldability of the udder and softness and mellowness of the gland tissue are generally accepted as indicative of good udder quality, and therefore are considered desirable. An abundance of fiber within the gland tissue of an udder is not ordinarily considered desirable, but does not necessarily indicate lack of quality. The amount of free space above the teat in each quarter might be considered as indicative of the size and character of the cistern. The amount of free space may be associated with udder quality because it is almost certain to be relatively greater in a loose, soft, and mellow udder than in a tight and hard one.

In evaluating the observations of udder quality, a grading system is used for each of the quality items, the grades ranging from a maximum of 9 to a minimum of 1. In each case a grade of 9 indicates the maximum of the characteristic under consideration, but does not necessarily signify excellence. For example, in the case of degree of fiber within the gland tissue, a grade of 9 would indicate extreme abundance of fiber regardless of whether a fibrous condition is desirable or objectionable. To illustrate the application of this grading system to characteristics ordinarily described in words, the nine grades for looseness and their word equivalents are given as follows: 9, extremely loose; 8, very loose; 7, loose; 6, medium to loose; 5, medium; 4, medium to tight; 3, tight; 2, very tight; and 1, extremely tight. The same grades can be applied to any of the items under consideration. As the free space can be measured in definite units, it is first recorded in inches. In order to permit the data for free space to be combined with grades for other items representing quality, the measurements of free space are converted into similar grades ranging between 1 and 9. Both inches and grades are given.

A combination of the grades for each of the five quality items is referred to as the composite grade for quality.

Ordinarily ante-mortem observations are made both before and after milking. The after-milking grades are used whenever they differ from the premilking grades. In all but three of the cases reported in this paper the ante-mortem observations were made within 16 days of the time of slaughter. The udder of cow 459 was examined 27 days before slaughter. At the time of slaughter cow 123 was just finishing the seventh month of a lactation period. The latest previous examination of her udder for quality was made late in the previous lactation period and is considered satisfactory for use. The latest examination of cow 272 was made when she had just finished the twelfth month of her first lactation and was nearly dry. She was not killed until 651 days later, when she was in an earlier stage of her third lactation and was producing 20 to 24 pounds of milk on one milking daily. Because of the difference in age, stage of lactation, and length of time between observation and slaughter, the observation grades for this cow are omitted.

The external form and general characteristics of the 11 udders as they appeared before the death of the cows are illustrated in Plate 1.

POST-MORTEM STUDIES OF THE AMPUTATED UDDER

In studying the size, capacity, and anatomy of the mammary gland in relation to its ante-mortem characteristics and its producing capacity, the udder is amputated immediately after the cow is killed and bled. After amputation the udder is attached to a frame in as nearly as possible a natural position and suspended by its surrounding skin and the median septum. If post-mortem milking tests are conducted they take place at this time. The empty weight of the amputated udder is then determined either by direct weighing or, when post-mortem milking tests are conducted, by deducting the weight of its contents from the total weight of the udder and contents. The secretory system of half of the udder is then filled by injecting 10 per cent formalin through the teats, the amount of fluid retained being used as a measure of the capacity of the secretory system of that half. After the half is filled the udder is divided into halves by cutting along the median septum. The filled half is frozen, cut with a saw into vertical transverse sections approximately 1 inch thick, and each section is photographed to furnish a permanent record of the gross anatomy of the gland at regularly spaced planes from its anterior to its posterior extremity. Blocks of tissue are taken from definitely located areas of both quarters of the untreated half for histological study.

Recovery of Milk from the Amputated Udder

The percentage of post-mortem recovery, or proportion of ante-mortem yield of milk obtained by milking the amputated udder, has been determined for each of the 11 cows discussed in this paper. The method employed in arriving at the percentage of post-mortem recovery was to determine over a period of 10 days the ante-mortem milking level of the cow. During this period the cow was milked, fed, and watered at a definite hour and every effort was made to maintain uniformity in her management. Slaughter took place on the eleventh

day, at the hour on which milking had taken place on previous days. As soon as the cow was stunned and bled the udder was amputated, suspended from a suitable frame in a natural position, and milked out by hand in the usual manner. Four hours later a second post-mortem milking took place for the purpose of removing the milk which had meanwhile drained into the cisterns and lower ducts. By dividing the total number of pounds of milk obtained in the first and second post-mortem milkings by the average number of pounds of milk obtained at corresponding ante-mortem milkings, the percentage of post-mortem recovery was obtained.

Size (Empty Weight) of Udder

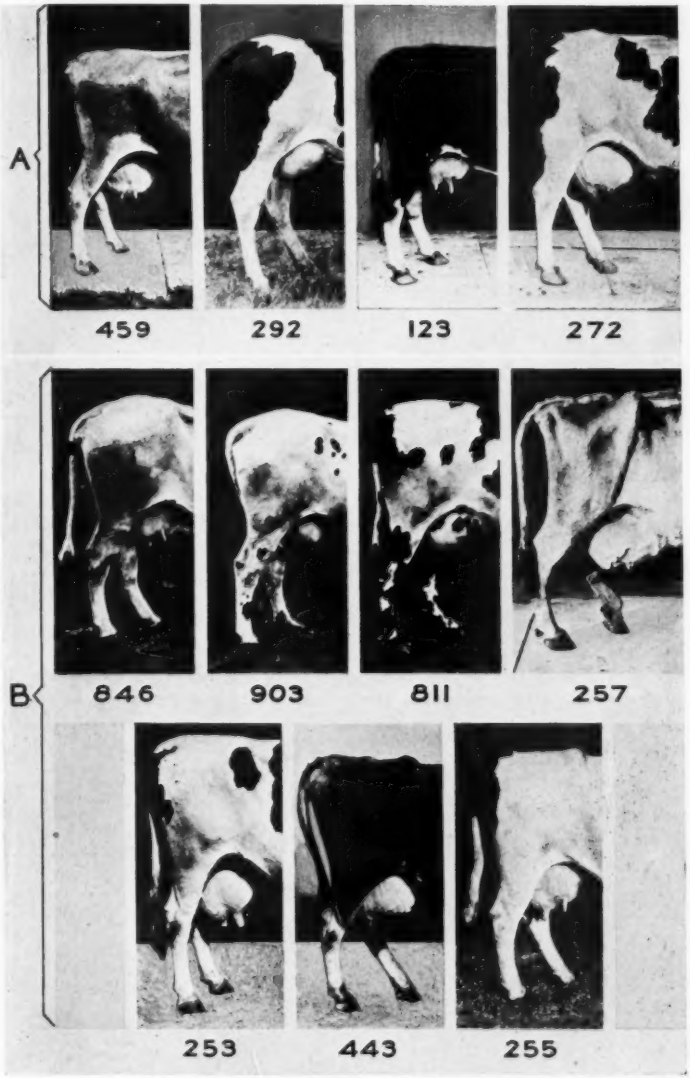
Size of udder is very difficult to determine in the living cow. Udders vary greatly in shape, and the definite landmarks necessary for measuring udder dimensions are not readily established. Furthermore, a determination of udder volume in the living cow by displacement would be attended with many difficulties. For example, it would be nearly impossible to make any equitable allowance for the degree of concavity of the upper surface of the udder, which is affected by variations in the inaccessible contour of the abdominal wall within the boundaries of the udder attachment. In these experiments each cow was slaughtered before being milked. The difference between the weight of the amputated udder in pounds before the post-mortem milking was performed and the total number of pounds of milk obtained at the first and second post-mortem milkings was used to represent the size or empty weight of the udder.

The retention of small quantities of milk, which in some cases was indicated after the second post-mortem milking, conceivably would introduce an error in the empty weight of udder as determined in this manner. Such an error in actual practice, however, was probably relatively slight and appears to have been unavoidable.

Capacity of Udder

As previously stated, the secretory system of half of the udder (usually the right half) was filled by injecting 10 per cent formalin through the teats, the quantity of formalin retained within this half of the udder being measured. Twice the quantity of formalin retained in the half of the udder filled in this manner gives the calculated volume capacity of both halves. This total volume in cubic centimeters is divided by 1,000 and multiplied by 2.2725 to give the "milk-equivalent capacity of the udder," or the weight in pounds of an equal volume of milk of average specific gravity. In the discussions which follow, "udder capacity" or "capacity of udder" refers to "milk-equivalent capacity of the udder," determined in this manner.

In the first 10 cases here reported, the formalin was injected by pressure obtained with a hand pump, but in filling the first 5 of these amputated udders the amount of pressure was not recorded. A gage was then attached to the pump to show the pressure at the point of injection (the teat). Accurate determination of the pressure by this method was difficult, for it might be 15 to 30 pounds on the stroke of the pump but would diminish as soon as the stroke was completed. The stability of pressure after cessation of pumping varied consider-



External appearance of the 11 udders included in this study. A, Group 1; B, group 2

A, Vertical transverse section through a rear quarter of udder 459; B, photomicrograph of tissue from udder 459

A, Vertical transverse section through a rear quarter of udder 292; B, photomicrograph of tissue from udder 292

A, Vertical transverse section through a rear quarter of udder 123; B and C, photomicrographs of tissue from udder 123—B, from normal-functioning quarter; C, from "blind" quarter

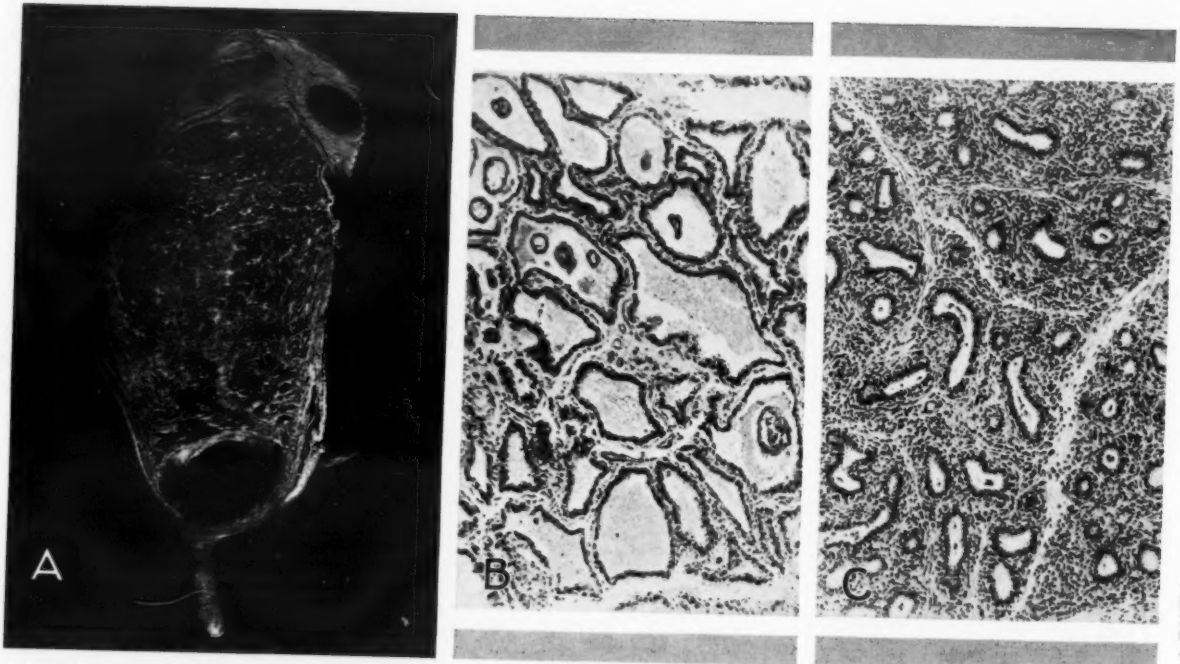
A, Vertical transverse section through a rear quarter of udder 272; B, photomicrograph of tissue from udder 272

A, Vertical transverse section through a rear quarter of udder 123; B and C, photomicrographs of tissue from udder 123—B, from normal-functioning quarter; C, from "blind" quarter

A, Vertical transverse section through a rear quarter of udder 272; B, photomicrograph of tissue from udder 272

A, Vertical transverse section through a rear quarter of udder 459; B, photomicrograph of tissue from udder 459

A, Vertical transverse section through a rear quarter of udder 292; B, photomicrograph of tissue from udder 292

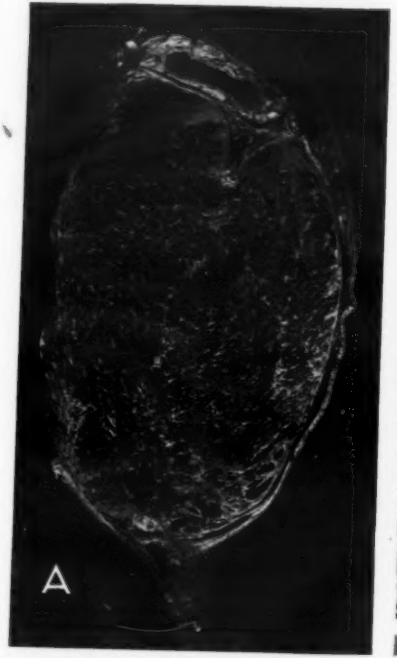


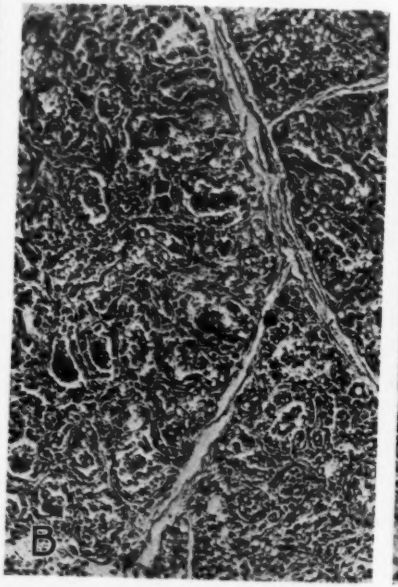
A, Vertical transverse section through a rear quarter of udder 123; B and C, photomicrographs of tissue from udder 123—B, from normal-functioning quarter; C, from "blind" quarter

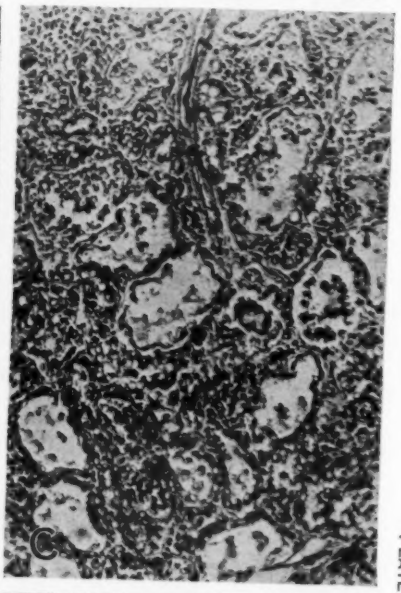
A, Vertical transverse section through a rear quarter of udder 272; B, photomicrograph of tissue from udder 272

A, Vertical transverse section through a rear quarter of udder 846; B, photomicrograph of tissue from udder 846

A, Vertical transverse section through a rear quarter of udder 903; B, photomicrograph of tissue from udder 903

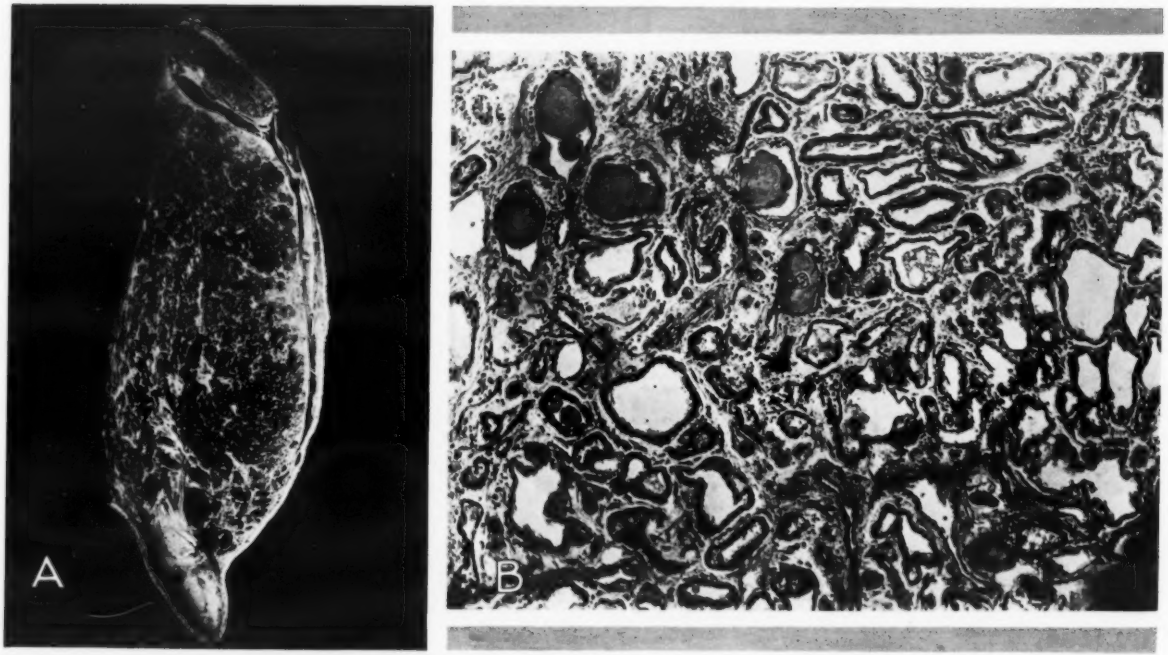






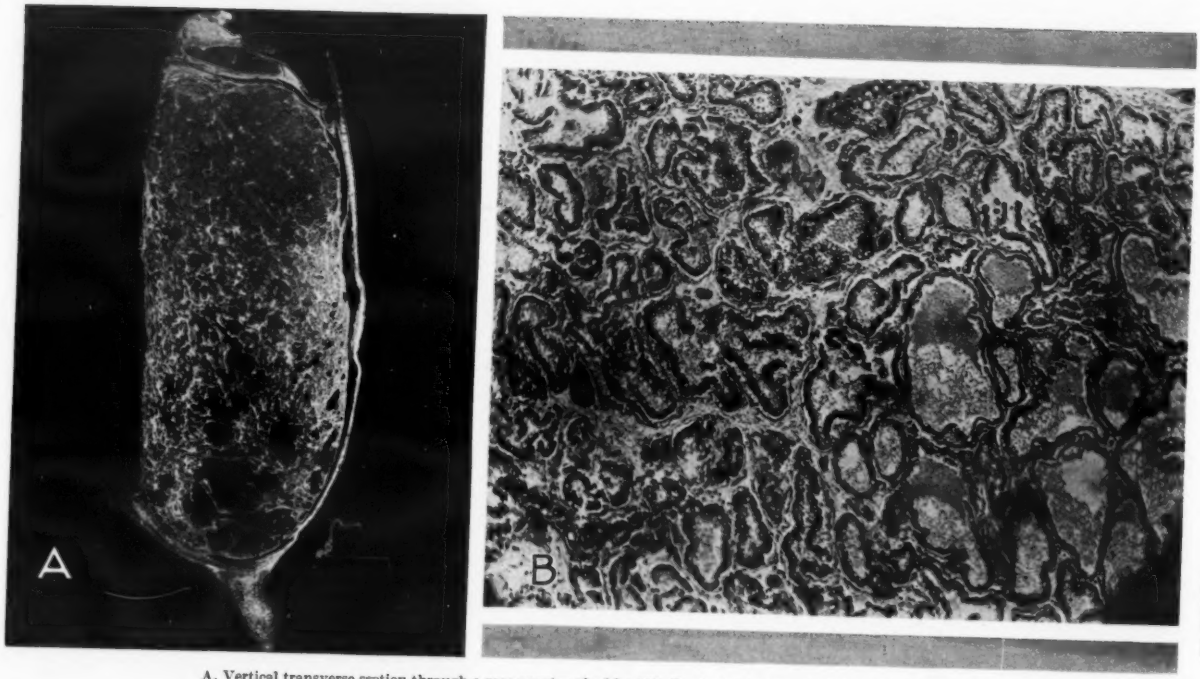
A, Vertical transverse section through a rear quarter of udder 811; B and C, photomicrographs of tissue from udder 811—B, from undersized quarter producing relatively small quantity of milk; C, from quarter recently affected by garget

A, Vertical transverse section through a rear quarter of udder 257; B, photomicrograph of tissue from udder 257



A, Vertical transverse section through a rear quarter of udder 253; B, photomicrograph of tissue from udder 253

A, Vertical transverse section through a rear quarter of udder 443; B, photomicrograph of tissue from udder 443



A, Vertical transverse section through a rear quarter of udder 255; B, photomicrograph of tissue from udder 255

ably with different udders. Pumping was continued until the udder was firm and well distended and the pressure had become fairly steady. For the next 5 udders the pressure became reasonably stable at 3½ to 6 pounds, or at an average of about 5 pounds. Later a tank was installed at an elevation sufficient to produce a gravity pressure of 10 pounds at the level of the udder, making it possible to obtain a constant pressure. About five minutes was required to fill an udder. The eleventh udder was filled by gravity pressure in the manner described.

Relation of Capacity to Weight of Udder

The "relation of capacity to weight of udder" expressed as a percentage is obtained by dividing the udder capacity by the weight of the empty udder and multiplying the result by 100. This relationship indicates the fluid-holding capacity of the secretory system for each 100 pounds of udder weight, and in a sense signifies the relative openness, porosity, or sponginess of the udder tissue.

Gross Anatomy

The gross anatomy of the cow's udder has been known in a general way for many years. Little is known, however, of the relation of the gross anatomy to the individual development, quality, and general characteristics of the udder, and the maximum producing capacity of the cow. A comparative study of this nature has been undertaken in the experiment-station herd of the Bureau of Dairy Industry at Beltsville, Md. Partial results are reported in connection with the 11 cows on which this report is based. The method of preparing and photographing consecutive vertical transverse sections from the extreme rear to the extreme front of one half of the udder has previously been outlined.[5] Plates 2 to 12, inclusive, show for each of the 11 lactating udders the gross anatomy of a section through the rear teat. A brief description of the gross structure of a rear quarter of each amputated udder appears in the discussion of the udder characteristics, life history, and performance of the individual cows.[6]

Great variations in type are shown in the illustrations of gross structure. Some of the udders have large, distinctly circumscribed and undivided cisterns, whereas others have very small cisterns which may be distinct in outline or which may consist only of a network of small communicating cavities. In some of the udders the tissue appears to be firm and dense; in others it is open and porous and the large ducts are distributed over a large proportion of the surface of the sections. Some of the udders have large quantities of connective tissue, appearing in white streaks; others have comparatively little.

Measuring any one of these three variables—relative size of cistern, openness of tissue, and proportion of connective tissue—in definite units would be particularly desirable, but obviously is difficult if not impossible. Some means of grading the udders or of comparing them with each other on the basis of these three variables is desirable in order that they may be correlated with udder quality characters, postmortem recovery of milk, size (empty weight) of udder, udder capacity, and milk-production records.

[5] Report of the Chief of the Bureau of Dairy Industry, U. S. Dept. of Agr., Dec. 11, 1929, p. 8.
[6] The age and stage of lactation of each cow studied are given in Table 1.

In the absence of a satisfactory method of recording these characteristics in definite units of measure, the 9-unit grading system previously described was used. Four men working independently assigned grades to each of the three variables, and a composite of the four individual ratings so obtained for each variable was prepared for use as a basis for determining the previously mentioned relationships between gross anatomy and the quality, capacity, and performance of the udder. Remarkable closeness in agreement was shown by those who graded the gross anatomy variables.

In addition to the grading of the gross-anatomy characteristics just described, an attempt was made to measure with a planimeter the size of the cistern. Photographs of two or more sections through a rear quarter of the udder were selected as representative. The photographs were the same as those used in assigning grades to the gross-structure characteristics. The cistern was measured as accurately as possible, then the area of the entire section was determined and the ratio of the former to the latter was calculated. On account of the very great variation in type and shape of cisterns, measurement of their areas was usually extremely difficult. Measurement of the distinctly circumscribed cistern, however, was comparatively simple even though its surfaces tapered so greatly that the areas at the two cut surfaces of a single section were in many cases greatly different from each other. Measurement of the divided cisterns, and in some cases this condition was extreme, was almost impossible. The measured and graded values checked very closely for the larger and more open cisterns. In general, however, those udders having divided or porous cisterns received a higher rating when graded visually than when measured, because during the visual examination allowance was automatically made for the area of the small cavities making up the cistern. On account of the indefiniteness of the shape and boundaries of these cavities, however, a measurement of many of them proved hardly feasible and was omitted.

Histology [7]

Although the general microscopic structure of the mammary gland has long been known, definite knowledge of the comparative histology of the udders of different cows, or the relation of histology of the udder to its individual characteristics and producing capacity is almost entirely lacking. A brief description of the histology of each of the 11 udders is presented in the discussion of udder characteristics, life history, and performance of the individual cows. In each case the discussion is based on a study of tissue selected from several definitely located areas in the half of the udder not treated with formalin. Plates 2 to 12, inclusive, show the histological appearance of the tissue from each of the 11 udders included in this study.[8]

PRESENTATION OF RESULTS

For the convenience of the reader the data showing the percentage of the ante-mortem production obtained by post-mortem milking [9] are repeated in Table 1, which also gives grades for quality, size

[7] The histological studies of the 11 udders were made by G. T. Creech.
[8] The age and stage of lactation of each cow studied are given in Table 1.
[9] Swett, W. W., Miller, F. W., and Graves, R. R. Op. cit.

(empty weight), capacity of secretory system, relation of capacity to weight, and gross anatomy ratings for the 11 udders included in the study. The first four cows constitute group 1. The next seven, which represent more carefully controlled conditions before and at the time of slaughter, and maintenance of body temperature in the amputated udders, are assembled in group 2.

SIGNIFICANCE OF UDDER QUALITY

To show the relationships which may exist between the quality grades assigned to udders on ante-mortem examination, and the characteristics of the same udders as determined after amputation, a number of coefficients of correlation [10] have been determined between the six udder-quality items given in Table 1 and the post-mortem recovery of milk, the size (empty weight) of udder, the capacity of udder, and the relation of capacity to weight of udder. It is unfortunate that data from a greater number of animals are not available for analysis in this study.

The degree of relationship between the individual udder-quality items and the other udder characteristics is shown by the magnitude of the simple correlations. A greater number of observations, of course, would have made possible a determination of the relationships with greater accuracy. The relation between any one udder-quality item and the post-mortem recovery of milk, for example, might be affected by the influence of any or all of the other udder-quality items. In order to make this study in the detail desired, net relationships by statistical methods would have to be determined, which would require establishing the extent of existing correlation for each pair of items, holding all other items constant. This has not been done, as the data available for this study are not sufficient to justify it.

Recovery of Milk From Amputated Udders

The degree of relationship between the ante-mortem udder-quality items listed in Table 1 and the post-mortem recovery of milk from the amputated udders is indicated by the correlation coefficients which follow:

Looseness of udder	$+0.7586 \pm 0.0905$
Yieldability of udder	$+ .7964 \pm .0780$
Softness and mellowness of gland tissue	$+ .4063 \pm .1781$
Abundance of fiber	$+ .5837 \pm .1406$
Average free space (inches)	$+ .6089 \pm .1342$
Composite grade for quality	$+ .8451 \pm .0610$

Every coefficient of correlation in this group is positive, and with one exception (softness and mellowness of gland tissue) every one is significant. It appears, therefore, that a definite relationship exists between the quality of the udder as judged on ante-mortem examination and the post-mortem recovery of milk. It seems reasonable to suppose that milk could be drawn more completely and more quickly from an udder of good quality than from one of poor quality.

[10] Throughout this paper, in presenting each coefficient of correlation a straight line relationship is assumed between the two variables.

TABLE 1.—*Udder quality of 11 cows and the relative post-mortem recovery of milk, size (empty weight), capacity, and gross anatomy of their amputated udders*

Cow No.	Breed of cow	Stage of lactation at time of—				Age at time of slaughter		Quality of udder as judged by ante-mortem examination							Milk obtained from udder		
		Ante-mortem examination		Slaughter				Looseness of udder	Yieldability of udder	Softness and mellowness of gland tissue	Abundance of fiber	Average free space		Composite of grades for quality	Ante-mortem	Post-mortem	Per cent of ante-mortem recovered post-mortem
Group 1		*Months*	*Days*	*Months*	*Days*	*Years*	*Months*	*Grade*	*Grade*	*Grade*	*Grade*	*Inches*	*Grade*	*Grade*	*Pounds*	*Pounds*	
459	Jersey	2	2	2	28	8	6	9	7	6	7	5.00	8	37	12.07	10.27	85.09
292	Holstein	1	12	1	12	2	3	5	6	7	4	2.75	4	26	21.38	10.60	49.58
123	Guernsey	12	11	6	28	4	3	8	7	7	7	2.83	4	33	10.37	7.20	69.43
272	Holstein			6	25	4	10								21.83	8.80	40.31
	Average														16.41	9.22	61.10
Group 2																	
846	Grade Holstein	3	25	4	1	6	2	9	9	9	7	5.00	8	42	18.51	18.70	101.03
903	do	13	27	14	8	5	2	8	7	7	7	4.81	8	37	16.27	11.60	71.30
811	do	23	16	24	1	6	9	7	7	5	7	4.25	7	33	15.20	9.70	63.82
257	Holstein	1	11	1	22	10	9	8	5	4	6	2.00	3	26	24.73	15.35	62.07
253	do	13	20	14	0	9	4	9	8	4	8	3.63	5	34	21.25	15.45	72.71
443	Jersey	6	8	6	19	9	4	8	8	6	7	3.31	5	34	17.41	15.20	87.31
255	Holstein	0	24	1	0	9	7	8	7	7	7	3.88	6	35	25.80	17.80	68.99
	Average														19.88	14.83	75.32
	Average of 2 groups							7.9	7.1	6.2	6.7	3.75	5.8	33.7	18.62	12.79	70.15

Cow No.	Breed of cow	Size (empty weight) of amputated udder	Capacity of udder			Relation of capacity to weight of udder	Gross anatomy			
			Portion of udder filled	Quantity of fluid held by portion filled	Milk equivalent		Relative size of cistern		Openness of tissue	Proportion of connective tissue
							Graded	Measured		
Group 1		*Pounds*	*Quarters*	*Cubic centimeters*	*Pounds*	*Per cent*	*Grade*	*Per cent*	*Grade*	*Grade*
459	Jersey	27.23	2 right	6,200	28.18	103.49	27.0	9.41	31	12
292	Holstein	44.40	2 right	10,200	46.36	104.41	17.0	3.81	19	6
123	Guernsey	23.30	2 left	7,660	34.81	149.47	25.5	7.45	16	10
272	Holstein	46.70	2 right	10,660	48.45	103.75	13.0	2.53	9	11
	Average	35.41		8,680	39.45	115.26				
Group 2										
846	Grade Holstein	25.80	2 right	13,800	62.72	243.10	24.0	6.42	20	6
903	do	36.90	2 right	14,390	65.40	177.24	9.0	.66	19	16
811	do	32.30	2 right	13,840	62.90	194.75	17.5	3.21	16	8
257	Holstein	54.15	2 left	12,030	54.68	100.98	5.5	2.19	16	9
253	do	44.80	2 right	11,880	54.00	120.54	13.5	5.59	11	20
443	Jersey	27.55	2 right	8,330	37.86	137.42	9.0	.89	8	20
255	Holstein	42.70	2 right	12,300	55.90	130.91	17.0	2.13	26	17
	Average	37.74		12,367	56.21	157.85				
	Average of 2 groups	36.89		11,026	50.11	142.36	16.2	4.03	17.4	12.3

Size (Empty Weight) of Udder

The degree of relationship between each of the ante-mortem udder-quality items, and the size (empty weight) of udder is shown by the following correlation coefficients:

Looseness of udder	−0.2868±0.1957
Yieldability of udder	−.6353±.1272
Softness and mellowness of gland tissue	−.5397±.1512
Abundance of fiber	−.3553±.1864
Average free space (inches)	−.5180±.1561
Composite grade for quality	−.6565±.1214

In contrast to the coefficients of correlation for post-mortem recovery of milk from the amputated udders, of which all are positive and all but one significant, those for size of udder are all negative, indicating an inverse relationship, and only two (yieldability of udder and composite grade for quality) are definitely significant. Two others (softness and mellowness of gland tissue and average free space) are of moderate or doubtful significance. Looseness of udder and abundance of fiber appear to have no relation to size of udder. A negative or inverse relationship between quality and size of udder is indicated.

Capacity of Udder

The degree of relationship between each of the udder-quality items and capacity of udder is shown by the following correlation coefficients:

Looseness of udder	−0.0488±0.2128
Yieldability of udder	+.0882±.2116
Softness and mellowness of gland tissue	+.0544±.2127
Abundance of fiber	+.0899±.2116
Average free space (inches)	+.2198±.2030
Composite grade for quality	+.1473±.2087

In this group one coefficient is negative and five are positive, but the probable error is greater than the coefficient, except in one case where they are nearly equal. None of the correlation coefficients in this group is of any significance whatever. Apparently quality and capacity were not in any way related in this group of udders.

Relation of Capacity to Weight of Udder

The degree of relationship between each of the ante-mortem udder-quality items and "relation of capacity to weight of udder", which is an index of its relative fluid-holding capacity or its porosity, is indicated by the following correlation coefficients:

Looseness of udder	+0.2112±0.2038
Yieldability of udder	+.6356±.1271
Softness and mellowness of gland tissue	+.5591±.1466
Abundance of fiber	+.3284±.1903
Average free space (inches)	+.5748±.1428
Composite grade for quality	+.6767±.1156

Every one of the coefficients in this group is positive. Three of them (yieldability of udder, average free space, and composite grade for quality) are distinctly significant, and another (softness and mellowness of gland tissue) might be classed as significant. The other two (looseness of udder and abundance of fiber) are not signifi-

cant. The similarity between the coefficients in this group and those for size (empty weight) of udder is striking, although all of those for relation of capacity to weight of udder are positive, whereas all of those for size of udder are negative. It appears that a number of the udder quality characteristics are positively correlated with relation of capacity to weight of udder.

In the four separate considerations of udder quality in relation to other udder characteristics, distinctly or moderately significant correlation coefficients were obtained for each of the quality items the following number of times: Looseness of udder, once; yieldability of udder, three times; softness and mellowness of gland tissue, twice; abundance of fiber, once; average free space, three times; and composite grade for quality, three times. The relation of quality grades in general to the percentage of post-mortem recovery of milk is the most significant, and their relation to the size of udder and to the relation of capacity to weight of udder are similar and relatively significant, but their relation to capacity of udder is of no significance.

FACTORS AFFECTING RECOVERY OF MILK FROM AMPUTATED UDDERS

The possible effect of management and method of killing the cows and the condition under which the amputated udders were kept, on the post-mortem recovery of milk, has been discussed in a previous paper.[11] The quantity of milk obtained from the amputated udders ranged from 7.20 to 18.70 pounds. The lowest and highest post-mortem recoveries were, respectively, 40.31 per cent and 101.03 per cent of the quantity obtained at corresponding ante-mortem milkings. (Table 1.) The degree of correlation between post-mortem recovery and ante-mortem udder quality grades is shown in a previous paragraph to be high. The coefficients showing the degree of correlation between the post-mortem recovery of milk and the size (empty weight) of udder, the capacity of udder, and the relation of capacity to weight of udder, follow:

Size (empty weight) of udder	-0.6750 ± 0.1107
Capacity of udder	$-.0516 \pm .2028$
Relation of capacity to weight of udder	$+.5611 \pm .1393$

The highest post-mortem recovery of milk (101.03 per cent) was obtained from the second lightest udder, the lowest post-mortem recovery (40.31 per cent) was obtained from the second heaviest udder, the second and third highest post-mortem recoveries were obtained from udders well below the average weight of udders included in this study, and the second and third lowest post-mortem recoveries were obtained from udders well above the average in weight. The udder of cow 903 weighed 36.90 pounds, which is almost the exact average weight for the entire group, and yielded 71.30 per cent, which is close to the average post-mortem recovery (70.15 per cent). In this group of udders, size is negatively or inversely correlated with post-mortem recovery, indicating that the small udder of good quality milks out most readily after amputation. No correlation was found to exist between capacity of udder and the readiness with which it released its milk post-mortem. The relation of capacity to weight of udder, however, appears to be positively

[11] SWETT, W. W., MILLER, F. W., and GRAVES, R. R. Op. cit.

correlated with post-mortem recovery or milking-out efficiency. A close relationship between quality of udder and relation of capacity to weight of udder is again suggested.

In considering the possible effect of the milking level immediately before slaughter on the recovery of milk from the udders after amputation, one might logically anticipate a lower percentage of post-mortem recovery from an udder producing at a high level than from one producing at a lower level just before slaughter. The ante-mortem production at a milking for the 11 cows studied ranged from 10.37 to 25.80 pounds and averaged 18.62 pounds. The cow having the lowest ante-mortem level (10.37 pounds) yielded 69.43 per cent on post-mortem milking. The one having the highest ante-mortem milking level (25.80 pounds) yielded almost the same proportion (68.99 per cent) on post-mortem milking. The sixth or middle cow in the group, from the standpoint of ante-mortem lactating level, whose ante-mortem production (18.51 pounds) was very close to the average for all the cows, yielded 101.03 per cent, which was by far the highest post-mortem recovery recorded for any cow. These facts might indicate little if any relation between ante-mortem producing level and post-mortem recovery. On the other hand, the second and third highest post-mortem recoveries (87.31 and 85.09 per cent) were from udders whose ante-mortem producing levels were below the average, and the two lowest post-mortem recoveries (40.31 and 49.58 per cent) were from udders whose ante-mortem producing levels were well above the average. The coefficient of correlation between the ante-mortem producing level of the 11 udders immediately before slaughter and the percentage of post-mortem recovery of milk from the same udders after amputation is -0.3579 ± 0.1773. This coefficient is negative but not significant. Apparently the percentage of post-mortem recovery was not materially influenced by the ante-mortem producing level.

SIGNIFICANCE OF SIZE (EMPTY WEIGHT) OF UDDER

The udders used in this study ranged in weight from 23.30 to 54.15 pounds, and averaged 36.89 pounds. The size, or empty weight of udder, has been shown in a previous paragraph to be negatively or inversely correlated with the characteristics ordinarily associated with udder quality. It also has been shown to be negatively or inversely correlated (-0.6750 ± 0.1107) with percentage of post-mortem recovery of milk from the udders after amputation. Undoubtedly a large udder is essential for liberal production of milk, as it has definitely been shown that most, if not all, of the milk obtained at a milking is present in the udder before milking is commenced. On the other hand, quality of udder is probably of considerable importance, as the smaller udder of good quality seems to be the one that yields its milk most readily on post-mortem milking. Quality may be shown by experiments now under way to indicate more rapid and complete milking in living cows. The question of a relationship between size of udder and capacity of udder should have a practical application and be of general interest. For this group of udders the coefficient of correlation between size and capacity is 0.3444 ± 0.1792, which is not significant. Apparently a relationship between these two factors does not exist. The coefficient of correlation between size and relation of capacity to weight of udder is -0.5631 ± 0.1389,

indicating that in this group the smaller udders have a greater capacity for each unit of weight than do the larger ones.

In a discussion of size or quality of udder, the matter of breed can not be entirely overlooked. The lighest udder was from a Guernsey, the second lightest was from a grade Holstein, and the third and fourth lightest were from Jerseys. The others were from either grade or registered Holsteins. The highest percentage post-mortem recovery of milk was from a grade Holstein, and the lowest was from a Holstein. The highest composite grade for udder quality was assigned to a grade Holstein, the next two highest grades to a Jersey and a grade Holstein, and the two lowest grades to Holsteins. The number of animals of each breed is so small that separate breed correlations could not be obtained. As far as its effect on the results covered up to this point is concerned, however, breed seems to be of little importance.

IMPORTANCE OF UDDER CAPACITY

The method of determining the capacity of the secretory system of cow udders by filling them with formalin and measuring the quantity of fluid they will hold, has been described in this paper. (p. 579.) The capacities of the 11 udders ranged from 28.18 to 65.40 pounds and averaged 50.11 pounds. Since the milk, as it is secreted, appears to be collected and held in the udder until it is withdrawn at milking time, it would appear that the capacity of the secretory system of the udder should be of definite importance.

The fact that most, if not all, of the milk is stored within the udder before milking is commenced, also raises the question of the importance of frequent milking of cows producing heavily. Results in practice indicate the necessity for more frequent milking of high-producing than of low-producing cows. On the other hand, the udder capacities given in Table 1 show that cow udders capable of holding 6 gallons of milk are not uncommon, and indicate ample space for storing in the udder all the milk obtained at a milking. In all probability, however, the udder of the living cow is not capable of holding a quantity of milk as great as the measured capacity of the amputated udder, because at the time of measuring, the blood has been removed from the udder, and because the pressure employed in filling it is probably greater than that of the milk within the living udder. A theory that has been advanced, which seems reasonable and which appears to be supported by scientific data, is that as the milk accumulates, the pressure within the udder gradually increases, and after a certain pressure has been developed, the rate of secretion decreases. In all probability secretion would be entirely stopped before a pressure as great as that used in filling the udder with formalin is reached. If the rate of secretion varies inversely with the accumulation of milk, the most rapid secretion should take place soon after milking and the greatest total secretion should take place in udders that are milked sufficiently often to keep the internal pressure below the point at which maximum activity of the secreting cells begins to be checked. This is in accord with the common practice of milking heavy producers more than twice daily, whereas two milkings daily seem to be sufficient for cows in moderate production.

The udder capacities given in Table 1 for this group of cows range from 28.18 to 65.40 pounds and average 50.11 pounds. Those for

udders 846, 903, and 811 may be a trifle high, as a layer of ice was found beneath the skin when they were sectioned, although not in sufficient quantity to affect materially the capacities given. The udder that was next to the smallest in size (empty weight) but highest in quality, in pounds of milk obtained post-mortem, and in percentage of post-mortem recovery, is very close to the highest in capacity. The three lowest capacities recorded were obtained for the two Jerseys and the Guernsey. Each of the three was below 40 pounds. All the Holsteins, both grade and registered, had udder capacities ranging from 46.36 to 65.40 pounds. It appears that in this study, which, however, includes but few animals, breed is more closely associated with results based on udder capacity than with results based on udder weight or udder quality. For an equivalent butterfat yield, the high percentage butterfat breeds would require less capacity for milk yield than the low percentage butterfat breeds. It seems probable, also, that an udder having a capacity sufficient to hold 50 pounds of 3 per cent milk could accommodate 50 pounds of 6 per cent milk, although the space required for the glands secreting the 6 per cent milk may or may not be greater than that required for the glands secreting an equal quantity of 3 per cent milk.

The capacity of the udder has already been shown to have no relation to the udder-quality grades. The relation of capacity to post-mortem recovery (-0.0516 ± 0.2028) also is of no significance. The correlation between capacity and size of udder (0.3444 ± 0.1792) is extremely doubtful if at all significant. Because of the method by which relation of capacity to weight of udder is determined, a significant and positive correlation between this ratio and udder capacity might be anticipated since capacity has a twofold influence. The coefficient in this case is found to be 0.5591 ± 0.1398.

SIGNIFICANCE OF RELATION OF CAPACITY TO WEIGHT OF UDDER

As previously stated, relation of capacity to weight of udder was determined by dividing the capacity of the udder by its weight and multiplying the result by 100. In every instance the capacity of the secretory system was greater than the weight of the udder. The percentages showing relation of capacity to weight of udder range from 100.98 to 243.10 and average 142.36. These percentages have been shown to be positively and rather definitely correlated with the udder-quality grades. Their correlation with post-mortem recovery of milk (0.5611 ± 0.1393) and with capacity of udder (0.5591 ± 0.1398) also are positive and significant, but their correlation with size (empty weight) of udder (-0.5631 ± 0.1389) is negatively or inversely significant. The positive correlation of these percentages with capacity of udder, and the similar negative correlation with size of udder, are more or less to be expected on account of the method of obtaining the "relation" percentages, which gives either capacity or weight a twofold influence in determining its correlation with relation of capacity to weight of udder. The positive and relatively significant correlations of relation of capacity to weight of udder with udder quality and with post-mortem recovery of milk indicate a fairly definite association between the three factors.

STAGE OF LACTATION

The stage of lactation of the cows used in this study varied from less than 1 month to more than 23 months when ante-mortem examinations were made, and from 1 month to 24 months when slaughtered. The influence of such a variation in stage of lactation on quality of udder, post-mortem recovery of milk, size (empty weight) of udder, and capacity of udder is logically questioned. It seems probable that the higher quality grades might be associated with advanced lactation, and since high quality and high post-mortem recoveries of milk are so definitely related, that the cows in advanced lactation would be the ones whose udders would milk out most completely post-mortem. Neither of these theories, however, is supported by the analysis of the data. The coefficient of correlation between stage of lactation and the composite grade for udder quality is 0.1280 ± 0.2098, and that between stage of lactation and percentage post-mortem recovery of milk is -0.0581 ± 0.2027. The decrease in magnitude of udder with advance in lactation is a common observation which would lead one to anticipate a negative relationship between stage of lactation and size of udder. The coefficient of correlation between these factors is negative but not significant (-0.1578 ± 0.1983). The coefficient of correlation between stage of lactation and udder capacity (0.4295 ± 0.1659) is of doubtful significance. On the whole, these results indicate that the wide variation in stage of lactation within this group of cows was not of great importance as a disturbing factor.

SIGNIFICANCE OF VARIATIONS IN UDDER STRUCTURE

A description of the method of grading the relative size of cistern, the openness of tissue, and the abundance of visible connective tissue, and of measuring the relative size of cistern with the use of a planimeter, has been given. The grades and percentages obtained are listed under "Gross anatomy" in Table 1. A study has been made to determine the relationship existing between each of these gross-anatomy variables and a selected few of the ante-mortem quality grades and post-mortem udder characteristics, the nature of which suggested the possibility of such a relationship.

Relative Size of Cistern (Graded)

A positive correlation between the relative size of cistern and some of the udder-quality grades, particularly the free space, has been assumed. It has seemed probable also that size of cistern might be associated with post-mortem recovery, capacity of udder, and relation of capacity to weight of udder but correlated negatively or inversely with size of udder. Coefficients of correlation have been determined to show the degree of relationship between the relative size of cistern (graded) and the following udder characteristics:

Yieldability of udder	$+0.0355 \pm 0.2130$
Softness and mellowness of gland tissue	$+ .5047 \pm .1590$
Average free space (inches)	$+ .6792 \pm .1149$
Composite grade for quality	$+ .4444 \pm .1712$
Per cent post-mortem recovery	$+ .3422 \pm .1796$
Size (empty weight) of udder	$- .6626 \pm .1141$
Capacity of udder	$- .3888 \pm .1726$
Relation of capacity to weight of udder	$+ .4224 \pm .1671$

The relative size of cistern (graded) appears to be distinctly and positively correlated with the average free space; its relation to softness and mellowness of gland tissue is moderate; its relation to composite grade for quality is of very doubtful significance; and its relation to yieldability of udder is of no significance. The relation of relative size of cistern (graded) to size (empty weight) of udder is, as anticipated, significantly negative or inverse, but its relation to percentage post-mortem recovery of milk, capacity of udder, or relation of capacity to weight of udder is of very doubtful significance.

Relative Size of Cistern (Measured)

A high degree of correlation between the grades for relative size of cistern and the corresponding percentages based on planimeter measurements would indicate that the results obtained by the two methods were similar. The coefficient of correlation is 0.8626 ± 0.0521. On account of the difficulties encountered in measuring the cisterns, and the greater possibility of allowance being made for the smaller divisions in the porous type of cistern when grading is done by visual examination, the grades are probably more nearly accurate than the percentages based on measurements. However, the following correlation coefficients have also been determined to show the degree of relationship between relative size of cistern (measured) and other udder characteristics.

Average free space (inches)	$+0.2554 \pm 0.1994$
Composite grade for quality	$+ .2930 \pm .1950$
Per cent post-mortem recovery	$+ .3520 \pm .1782$
Size (empty weight) of udder	$- .4773 \pm .1570$
Capacity of udder	$- .5004 \pm .1525$
Relation of capacity to weight of udder	$+ .0316 \pm .2032$

A low negative or inverse correlation with both size and capacity of udder is suggested, but the others are not significant.

Openness of Tissue

It seems reasonable to suppose that openness of tissue would be positively correlated with udder quality, percentage of post-mortem recovery of milk, capacity of udder, and relation of capacity to weight of udder, but negatively or inversely correlated with size of udder. The relationships found to exist are shown by the following coefficients of correlation.

Yieldability of udder	-0.1546 ± 0.2082
Softness and mellowness of gland tissue	$+ .3617 \pm .1854$
Average free space (inches)	$+ .3604 \pm .1856$
Composite grade for quality	$+ .2700 \pm .1977$
Per cent post-mortem recovery	$+ .3437 \pm .1793$
Size (empty weight) of udder	$- .2004 \pm .1952$
Capacity of udder	$- .1131 \pm .2008$
Relation of capacity to weight of udder	$- .0364 \pm .2031$

In every instance in this group, the coefficient is less than twice the probable error. It appears, therefore, that the openness of tissue as graded for this group of udders, is not correlated with udder quality or with any of the other udder characteristics studied.

Proportion of Connective Tissue

"Proportion of connective tissue" refers to the relative abundance of light-colored streaks of tissue which are visible on the cut surfaces of the gross-anatomy sections of the amputated udders. "Abundance of fiber," which has frequently been discussed in connection with udder quality, refers to a condition that is observed at the time of the ante-mortem examination of the udder. It is described in detail at the beginning of this paper. Although the proportion of connective tissue and the abundance of fiber in an udder are judged by entirely different methods, one would anticipate a fairly close relationship between these two factors. Perhaps this so-called connective tissue that is seen in the sectioned udder and the so-called fiber that is felt on examination of the udder are one and the same thing. Plate 11, A, a photograph of a vertical transverse section of the udder of cow 443, is a very good illustration of the appearance of connective tissue in the gross-anatomy sections. It appears here as white streaks resembling somewhat the fatty marbling of beef. The photomicrograph of tissue from the udder of the same cow (pl. 11, B) is a good illustration of the minute structure of connective tissue. It has the appearance of a river, the main stream of which runs from the middle of the picture at the right, in a big bend toward the bottom and back toward the center at the left. In order to determine the relation between the proportion of connective tissue appearing on the cut surface of the gross-anatomy sections and the quality and other characteristics of the udder, the following correlations were derived.

Yieldability of udder	$+0.2884 \pm 0.1956$
Softness and mellowness of gland tissue	$-.3126 \pm .1925$
Abundance of fiber	$+.5800 \pm .1415$
Composite grade for quality	$+.2020 \pm .2046$
Per cent post-mortem recovery	$+.1983 \pm .1954$
Size (empty weight) of udder	$+.0432 \pm .2030$
Capacity of udder	$-.0980 \pm .2014$
Relation of capacity to weight of udder	$-.2394 \pm .1917$

A fairly significant positive correlation is found to exist between proportion of connective tissue and abundance of fiber. All the other correlations, however, are not significant.

In the entire group of 30 correlations based on grades for gross-anatomy characteristics, only 3 could be considered as definitely significant. Positive correlations are found between size of cistern (graded) and average free space, and between proportion of connective tissue and abundance of fiber. A negative correlation is found between size of cistern (graded) and size of udder. The ability to anticipate accurately the gross structure of the udder by examining it ante-mortem appears from these results to be very limited.

Relation of Udder Characteristics to Producing Capacity

The ultimate goal of this general study of the udder is to determine for a large number of cows the relationship of the conformation, quality, gross anatomy, and histology of the udder to its milk and butterfat producing capacity. The data are presented here to complete the information given in connection with the 11 cows used in the post-mortem milking experiments. Data similar to those presented in this paper are being secured on a considerable number of

cows of known producing ability with records made under comparable conditions at the Beltsville experiment station. Unfortunately the production records of these 11 cows were not all made under uniform conditions. The production records were made at ages varying from 2 to 6 years. Moreover, the record of one cow was not complete. In general the milk and butterfat records were made under official testing conditions on three daily milkings, but three of the cows were obtained from another herd where milk production only was recorded and where the cows were kept under average herd conditions and milked twice daily.

TABLE 2.—*Producing capacity of 11 cows included in this study* [a]

Cow No.	Breed	Record of production for 1 year								
		Milk	Butterfat	Age of cow		Times milked daily	Mature equivalent			Numerical rating on production [b]
							Milk	Butterfat	Milk with additional correction for management	
		Pounds	*Pounds*	*Years*	*Months*		*Pounds*	*Pounds*	*Pounds*	*Grade*
459	Jersey	10,153	536	2	1	3	[c] 13,909	[c] 712	13,909	6
292	Holstein	[d]								
123	Guernsey	8,535	477	2	5	3	10,412	581	10,412	5
272	Holstein	12,762	447	2	0	3	17,866	625	17,866	5
846	Grade Holstein	12,220		4	9	2	12,831		19,246	6
903	do	12,943		3	11	2	14,366		21,549	7
811	do	10,928		4	9	2	11,474		17,211	5
257	Holstein	24,135	852	6	4	3	24,135	852	24,135	8
253	do	16,405	628	5	8	3	16,569	634	16,569	5
443	Jersey	9,621	465	6	4	3	9,621	465	9,621	4
255	Holstein	11,202	415	2	8	3	[c] 11,202	[c] 415	11,202	3

[a] The arrangement of data in this table is the same as in Table 1.

[b] As explained in the following pages, the grades in the last column give each cow credit for her production record on the basis of the performance that could be expected of her when breed characteristics are taken into consideration.

[c] See comment on record following this table.

[d] Record not completed.

In order to place the records as nearly as possible on a comparable basis, adjustment was first made for variations in age by determining the mature equivalent of each production record as made. The age adjustments were made on the basis of factors determined by Fohrman,[12] some of which are as yet unpublished. The milk and butterfat records as made and their mature equivalents are given in Table 2. One point which might otherwise be considered a discrepancy deserves an explanation. The highest production record of cow 459 was made at 2 years 1 month of age. Although she lived to make other records, the mature equivalent is determined on the basis of the first record because she developed a pathological condition in the genital organs which rendered her apparently incapable of subsequent normal reproduction or maximum lactation. The record made at 2 years 1 month of age is, therefore, considered the most accurate index of this cow's inherent producing capacity. On the other hand, cow 255

[12] FOHRMAN, M. H. FACTORS FOR ADJUSTING MILK AND BUTTERFAT RECORDS OF REGISTER OF MERIT JERSEY COWS TO A UNIFORM AGE BASIS. Jour. Dairy Sci. 9: 469–480, illus. 1926.

made several subsequent records and never improved that recorded at 2 years 8 months of age. Since she failed to show any physical condition which might explain her failure to improve the first-lactation performance, it is used also as her mature equivalent without adjustment. Woodward [13] has shown that cows kept under official-test conditions and milked and fed three times daily produce approximately 50 per cent more milk and butterfat than do the same cows when kept under average herd conditions. The milk-production records of cows 846, 903, and 811, which were made on two milkings daily and under more or less average herd conditions, were again adjusted to allow for conditions of management by the addition of 50 per cent to their mature equivalents. The adjusted records for these three cows together with the mature equivalent milk records for the others are presented in a separate column in Table 2. As a basis for studying the relation of the characteristics of the udder to the quantity of milk produced by it, these data are the best obtainable.

Coefficients of correlation have been determined to show the relation between each of the 14 udder characteristics under consideration and the milk production of the cow expressed in pounds, as finally adjusted for age and conditions of management. These correlation coefficients, with the udder characteristics designated, follow:

Looseness of udder	+0.0200±0.2247
Yieldability of udder	−.3484±.1975
Softness and mellowness of gland tissue	−.2076±.2151
Abundance of fiber	−.3704±.1940
Average free space (inches)	+.0229±.2247
Composite grade for quality	−.1665±.2186
Per cent post-mortem recovery	−.2168±.2033
Size (empty weight) of udder	+.5467±.1496
Capacity of udder	+.6379±.1265
Relation of capacity to weight of udder	+.1406±.2091
Relative size of cistern (graded)	−.4017±.1789
Relative size of cistern (measured)	−.2311±.2019
Openness of tissue	−.0526±.2127
Proportion of connective tissue	−.4071±.1779

In this group of 14 items the coefficient is less than its probable error in 6 cases, less than twice the probable error in 4 other cases, and less than three times the probable error in 2 other cases. None of the quality grades shows any significant correlation to production of milk. In only two instances, capacity of udder and size of udder, is the correlation significant, and the latter is only moderately so. These correlations of both capacity of udder and size of udder to milk-producing ability indicate that the high producer possesses an udder of greater size and capacity than does the low producer. This does not necessarily mean that the size or capacity of the udder is proportional to the total yearly production, as a large total yearly production record may result from a high maximum with a relatively rapid decline, or from a more moderate maximum and greater persistency. The correlations shown, however, indicate that the udder must be of sufficient size and capacity to hold the amount of milk secreted between milkings. This is in harmony with previous observations of the writers [14] that most, if not all, of the milk obtained at a milking is held in the udder before the milking process is commenced.

[13] WOODWARD, T. E. INFLUENCE OF TWO PLANES OF FEEDING AND CARE UPON MILK PRODUCTION. Jour. Dairy Sci. 10: 283–291. 1927.

[14] SWETT, W. W., MILLER, F. W., and GRAVES, R. R. Op. cit.

The foregoing consideration of the relation of udder characteristics to producing capacity was based entirely on quantity of milk produced and made no allowance whatever for butterfat production or for breed characteristics. A quantity of milk that would represent high production for a Jersey or Guernsey would be considered close to average for a Holstein, and the two Jerseys and the one Guernsey were all located in the lowest four positions in the alignment on which the previous consideration was based. Undoubtedly some of these cows occupy positions considerably below what they would have if butterfat production and breed characteristics had been considered.

It is desirable that a similar study should be made of the relation of quality grades and other characteristics of the udder to producing capacity, in which each cow is given credit for production on the basis of the performance that could be expected of her if breed characteristics were taken into consideration. Such credits could be based with reasonable fairness on butterfat production if the actual butterfat production were known in all cases. For three of the cows the percentage of butterfat was not determined when the milk records were made. Although the approximate richness of their milk was known, and their butterfat production could be approximated, in view of the lack of definite records of butterfat production it was thought best to use the grading system instead of basing the correlation coefficients on the butterfat yield. The results would probably be essentially the same in either case.

The 9-point grading system described in this paper was employed. With primary attention directed to the known and estimated butterfat production, but with the milk production records and the breed characteristics of each cow in mind, the cows were placed in one of six production groups ranging from very high to low, and each cow was assigned an equivalent numerical grade. The grades assigned appear in the last column of Table 2. The six groups used and their equivalent numerical grades are as follows: Very high production, 8; high production, 7; medium to high production, 6; medium production, 5; medium to low production, 4; and low production, 3.

Coefficients of correlation have been determined to show the degree of relationship between the 14 udder characteristics under consideration and the numerical ratings assigned to represent the production performance of each cow. Udder characteristics are listed below in the order in which they appear in Table 1, and each is followed by the coefficient of correlation indicating the extent of relationship with the performance grades.

Looseness of udder	$+0.1381 \pm 0.2205$
Yieldability of udder	$-.4166 \pm .1858$
Softness and mellowness of gland tissue	$-.1765 \pm .2178$
Abundance of fiber	$-.4969 \pm .1693$
Average free space (inches)	$-.0703 \pm .2237$
Composite grade for quality	$-.2174 \pm .2142$
Per cent post-mortem recovery	$+.0039 \pm .2133$
Size (empty weight) of udder	$+.2348 \pm .2015$
Capacity of udder	$+.2059 \pm .2043$
Relation of capacity to weight of udder	$+.0337 \pm .2131$
Relative size of cistern (graded)	$-.2279 \pm .2022$
Relative size of cistern (measured)	$+.0620 \pm .2125$
Openness of tissue	$+.0878 \pm .2116$
Proportion of connective tissue	$-.4426 \pm .1715$

Apparently not one of the characteristics of the udders studied is distinctly indicative of the cow's ability or lack of ability to perform, as shown by the performance ratings. In 7 of the 14 items the coefficient is less than its probable error; in 4 other cases it is less than twice its probable error; and in the 3 remaining items it is less than three times its probable error, although 1 is very close to the ratio of 3 to 1. The coefficient for abundance of fiber is of only moderate significance and might be indicative of the undesirability of abundant fiber in the udder. It is worthy of note also that the next highest coefficient is for proportion of connective tissue. The abundance of fiber was judged in the living cow. The proportion of connective tissue was judged from the photographs of the gross-anatomy sections. The fact that these two correlations are both negative and that they are the highest in the entire group might be interpreted as indicating that, as has been assumed, the fiber of the living udder and the visible connective tissue in the gross sections are either closely related or are the same, and also that they are not beneficial and may be detrimental to high production.

Both size of udder and capacity of udder were found to be fairly definitely correlated with the cow's ability to produce milk, but were not correlated with the performance grades which give consideration to butterfat production. This fact suggests again the comment made under the discussion of udder capacity, that for an equivalent butterfat yield the breeds having a high butterfat test would require less capacity for milk yield than the breeds having a low butterfat test.

DISCUSSION OF THE UDDER CHARACTERISTICS, LIFE HISTORY, AND PERFORMANCE OF EACH COW

COW 459 (PRODUCTION GRADED AS MEDIUM TO HIGH)

Cow 459 was a registered Jersey. Her record of 10,153 pounds of milk and 536 pounds of butterfat was made under official testing conditions at the age of 2 years 1 month. She failed to equal this record again, but on account of the fact that she developed a pathological condition which rendered her apparently incapable of subsequent normal reproduction or maximum lactation, the mature equivalent of this 2-year-old record, which amounts to 13,909 pounds of milk and 712 pounds of butterfat, was used as a measure of her producing capacity. She was slaughtered in the third month of lactation at 8 years of age. Her udder was very high in composite grade for quality, relatively low in size (empty weight), had the smallest capacity in the group, a very low relation of capacity to weight of udder, and yielded a high percentage of ante-mortem production on post-mortem milking.

Gross anatomy.—This udder shows a very large, open, and rather definitely circumscribed cistern. The gland tissue is very open and porous, and the relatively large ducts are distributed over nearly all the surface of the sections. An average quantity of connective tissue is visible.

Histology.—Sections from a front quarter of the udder showed many of the alveoli to be in a state of functional inactivity in this portion of the gland. There was an abnormal amount of fibrous tissue in this quarter and many of the lobules showed deposits of numerous small concretions, or milk calculi, in the alveoli. The histological

appearance of the rear quarter indicated normal functional activity of this portion of the gland. In certain areas there was some loosening and sloughing of the epithelial lining of the acini, and a few small inflammatory centers were noted. The area illustrated in Plate 2 was taken from the central part of the left rear quarter, which was functioning but which was noticeably smaller and narrower than the other quarters.

General.—This cow was a persistent aborter. At the last five parturitions one calf was born that died at 2 days of age and the other four were abortions of single or twin fetuses. She bred normally, but apparently was unable to retain a fetus after it reached a certain size. This probably accounts for the fact that her maximum production was made at 2 years of age, for the record indicated is sufficient to show beyond doubt that she had an inheritance for good production. Available records fail to show any udder disturbance requiring treatment. The possible influence of abortion on either gross anatomy or histology of the udder is entirely speculative. The significance of age on structure also is speculative. The large cistern and open structure of gland tissue may have been at least partly responsible for the relatively high post-mortem recovery of milk.

COW 292 (PRODUCTION NOT GRADED)

Cow 292, a registered Holstein, did not have a complete yearly record of production, but she was lactating at an average 2-year-old level when slaughtered in the second month of lactation at 2 years 3 months of age. This cow was killed for this work, because she did not fit into the breeding projects and space was needed. Her udder was relatively very low in composite grade for quality, relatively high in size (empty weight), less than average in capacity, low in relation of capacity to weight of udder, and very low in percentage post-mortem recovery of milk.

Gross anatomy.—This udder has an average sized and distinctly circumscribed cistern. The gland tissue is open in the lower part, relatively dense above and of nearly average porosity as a whole. The visible connective tissue is very limited in quantity.

Histology.—The appearance of this gland, for the most part, indicated normal functional activity. One section from the central area of a rear quarter showed some evidence of chronic inflammatory changes, as indicated by a perceptible increase in the interstitial tissue in the areas involved. The fibrous framework of the gland in sections from normal areas was unusually soft or loose in structure. The area illustrated in Plate 3 was taken from a point near the base and about midway between the front and rear quarters on the left side.

General.—Examination of the living udder indicated that it was normal, except that the right rear quarter was slightly larger than the others. There is no record of this cow's having had udder disturbances requiring treatment. An udder at this age would hardly be expected to show excessive connective tissue, and should be actively functioning at this stage of lactation. Relationships between producing ability and udder characteristics are not obtainable, owing to the early age at which the cow was slaughtered.

COW 123 (PRODUCTION GRADED AS MEDIUM)

Cow 123, a registered Guernsey, produced 8,535 pounds of milk and 477 pounds of butterfat under official testing conditions at the age of 2 years 5 months. The maturity equivalent based on this production is 10,412 pounds of milk and 581 pounds of butterfat. She was slaughtered in the seventh month of lactation at the age of 4 years 3 months. Her udder was about medium in composite grade for quality, the lowest in the entire group in size (empty weight), next to lowest in capacity, about average in relation of capacity to weight of udder, and almost exactly average in percentage post-mortem recovery of milk.

Gross anatomy.—This udder has a very large and unusually even and distinctly circumscribed cistern above which the gland tissue is of about average density and somewhat less than average in proportion of visible connective tissue. After treatment with formalin and sectioning, the tissue was flexible, soft, watery and almost mushy.

Histology.—In sections from a front quarter of the udder the alveoli were seen to be well developed, indicating normal functional activity. Some evidence of degeneration of the glandular epithelium was noted in several of the sections. All the sections from the rear quarter showed an inactive state of the glandular tissue. The various lobules showed a marked increase in the interlobular connective tissue of the quarter. On account of the entirely different conditions existing in the right rear as compared with the other quarters (see next paragraph), the histological structure of both the right front (functioning) and the right rear (inactive) quarter is illustrated in Plate 4.

General.—The right rear quarter was indurated and entirely inactive and was described as relatively small and consisting only of a fibrous cylinder extending toward the teat. Undoubtedly this accounts for the histological notations for that quarter. Apparently the other quarters were functioning normally. There is no other record of this cow's having had udder disturbances requiring treatment.

COW 272 (PRODUCTION GRADED AS MEDIUM)

Cow 272, a registered Holstein, made a production record of 12,762 pounds of milk and 447 pounds of butterfat at 2 years of age on three milkings daily. This is equivalent to a mature production of 17,866 pounds of milk and 625 pounds of butterfat. She was in the seventh month of lactation and was 4 years 10 months of age at the time of slaughter. She was destroyed at this early age because she had been pronounced permanently sterile. Observations on the living udder are not available for this cow. The amputated udder was one of the largest in size (empty weight) of those examined, was almost average in capacity, very low in relation of capacity to weight of udder, and yielded the lowest percentage of ante-mortem production on post-mortem milking.

Gross anatomy.—In size of cistern this udder ranks considerably below the average. The teat canal and cistern are continuous and well circumscribed. The tissue is very dense and large ducts are confined to a small area above the cistern. An average quantity of fairly well distributed connective tissue is visible. The ducts were white and shiny, and the gland tissue after formalin treatment and sectioning was soft, wet, flabby, and sloppy.

Histology.—Histological examination of this udder showed a mixture of functioning and nonfunctioning lobules of the gland in the different areas sectioned. There was a limited increase in the fibrous connective tissue of the organ. A number of milk concretions were seen in different lobules. The histological area illustrated in Plate 5 was taken from a point near the base of the left front quarter.

General.—Available records failed to indicate any udder disturbance requiring treatment during a previous lactation, but the left front quarter was described as small. During the preliminary 10-day milking period immediately before slaughter, however, a hardened and congested condition developed in the left front quarter which was accompanied by a greatly reduced flow of milk from that quarter. After two days of treatment by massaging, the condition of the udder and the quantity of milk appeared to be about normal but the milk was ropy. After three days of treatment the udder and the milk appeared to be entirely normal.

COW 846 (PRODUCTION GRADED AS MEDIUM TO HIGH)

Cow 846, a grade Holstein, produced 12,220 pounds of milk at 4 years 9 months of age on two milkings daily. The mature equivalent of this record is 12,831 pounds, and an additional adjustment for management raises it to 19,246 pounds. This cow was slaughtered in the fifth month of lactation at 6 years 2 months of age. Her udder was very good to excellent in quality, ranking first in the group on the basis of composite grade. It was next to the smallest in size (empty weight), close to the highest in capacity, and by far the highest in relation of capacity to weight of udder. It also yielded by far the highest proportion of ante-mortem production on post-mortem milking.

Gross anatomy.—The cistern is large and definite in outline with many large ducts leading from it. The tissue is above the average in openness and porosity and has very little visible connective tissue. The gland tissue after treatment with formalin and sectioning was soft and flexible and very wet.

Histology.—Sections from this udder showed a rather heavy fibrous framework, the interstitial connective tissue being soft or loose in texture. A few of the lobules in both front and rear quarters showed a state of inactivity, but for the most part this gland appeared to have been functioning normally. The area illustrated in Plate 6 was taken from a point near the base of the left front quarter.

General.—The history of udder disturbances for this cow is lacking, but the ante-mortem examination failed to show any abnormality or lack of uniformity in condition indicative of such disturbances.

COW 903 (PRODUCTION GRADED AS HIGH)

Cow 903, a grade Holstein, produced 12,943 pounds of milk at about 3 years 11 months of age on two milkings daily. The mature equivalent of this production is 14,366 pounds for the conditions under which it was made, or 21,549 pounds when adjusted for conditions of management. This cow was slaughtered in the fifteenth month of lactation at 5 years 2 months of age. Her udder was rated very high in composite grade for quality, very close to average in size (empty weight), the highest of the group in capacity, near the

top in relation of capacity to weight of udder, and remarkably close to average in percentage post-mortem recovery of milk.

Gross anatomy.—The cistern consists of a honeycomblike mass of very small and more or less independent cavities, is indefinite in outline and appears to be small in total volume. This udder is above the average in openness of structure of gland tissue and shows more than the average quantity of connective tissue. After treatment with formalin and sectioning, the gland tissue was soft, flexible, very wet, and sloppy. For several days after the sections were cut a milky white exudate continued to flow from the cut surface of the tissue.

Histology.—The fibrous framework of this udder was found to be very soft or loose in texture. There was considerable degeneration of the epithelial tissue, and in many of the lobules there was sloughing of the epithelial lining of the alveoli. In places one or more of the alveoli had been completely destroyed and in some areas they had apparently been replaced by fat tissue. A section from the superior portion of the rear quarter showed groups of inactive lobules. Groups of alveoli partly or completely filled with pus cells were seen in a number of the sections. The area illustrated in Plate 7 was taken from a point near the base of the left front quarter.

General.—The history of udder disturbances for this cow is lacking, but ante-mortem examination failed to show evidence of such disturbance, abnormality, or lack of uniformity in condition except that the udder was "relatively light" in the left rear quarter.

COW 811 (PRODUCTION GRADED AS MEDIUM)

Cow 811, a grade Holstein, produced 10,928 pounds of milk on two milkings daily at about 4 years 9 months of age. The mature equivalent of this production is 11,474 pounds for the conditions under which it was made, or 17,211 pounds if adjusted for conditions of management. She was slaughtered about 24 months after her last calving, at the age of 6 years 9 months. Her udder was only slightly below the average in composite grade for quality, slightly below the average in size (empty weight), next to the highest in the group both in capacity and in relation of capacity to weight of udder, and considerably below the average in percentage of ante-mortem production recovered on post-mortem milking.

Gross anatomy.—The cistern is about average in size, somewhat divided, and indistinct in outline. The gland tissue appears to be of average openness of structure and to have only a small quantity of connective tissue. The udder tissue was generally very flexible, soft, and watery when sectioned.

Histology.—This udder showed evidence of infection. Many of the alveoli and a number of the milk ducts were filled with pus cells. There was some degeneration and sloughing of the alveolar epithelium, and the sections from the various areas showed an increase in the interlobular connective tissue. Many of the alveoli were filled with concretions, pus cells, and cell detritus. Because of the abnormal conditions in the left front quarter (see next paragraph), histological sections from both front and rear quarters are illustrated in Plate 8.

General.—The previous history of udder disturbances for this cow is lacking, but when the cow was obtained for this study the left

rear quarter of her udder was noticeably small and stringy, and produced less milk than the other quarters, which were relatively full and round. A few days before she was slaughtered garget developed in the left front quarter, and the milk from that quarter diminished in quantity and became thick and ropy. In all probability this accounts for the presence of pus cells and to some extent explains the other indications of degeneration noted in connection with the histological examination of the udder.

COW 257 (PRODUCTION GRADED AS VERY HIGH)

Cow 257, a registered Holstein, produced 24,135 pounds of milk and 852 pounds of butterfat under official-testing conditions at the age of 6 years 4 months. A previous record made under similar conditions at 3 years 1 month of age amounted to 20,357 pounds of milk and 702 pounds of butterfat. She was slaughtered in the second month of lactation at the age of 10 years 9 months. Her udder was very low in composite grade for quality, the largest in size (empty weight) in the group, considerably above the average in capacity, the lowest in relation of capacity to weight, and considerably below the average in percentage of ante-mortem production recovered on post-mortem milking.

Gross anatomy.—A few ducts of medium size are present, but the quarter shown is almost devoid of a definitely formed cistern. The volume of the cistern or the few ducts above the teat is extremely small. The structure of the gland tissue is of about average openness and the amount of visible connective tissue rather small. The tissue after treatment was spongy, rubbery, and fairly typical. The structure was unusually uniform in all the sections of this udder.

Histology.—This udder showed chronic inflammatory changes, as indicated by extensive round cell infiltration and a noticeable increase in the interalveolar and interlobular connective tissue. The increase in the interstitial tissue resulted in atrophic changes or decrease in the number and size of many of the alveoli. Many of the acini showed evidence of inactivity. In this connection the advanced age and heavy production of this cow should be taken into consideration. The area illustrated in Plate 9 was selected from a point near the base of the right rear quarter of the udder.

General.—An examination of records failed to show any disturbance during the life of the cow requiring treatment of the udder. The udder appeared to be normal and was functioning in all quarters. The ante-mortem examination indicated the presence of fiber which was relatively abundant and harsh, especially in the rear quarters, and no evidence of open cisterns. These observations are supported by the histological findings which indicate abundant connective tissue, and by the fact that the gross-anatomy sections showed an extremely small cistern.

COW 253 (PRODUCTION GRADED AS MEDIUM)

Cow 253, a registered Holstein, produced 16,405 pounds of milk and 628 pounds of butterfat under official testing conditions at the age of 5 years 8 months. The mature equivalent of this record would be 16,569 pounds of milk and 634 pounds of butterfat. She was slaughtered 14 months after her last calving, at the age of 9 years

4 months. Her udder was about average in composite grade for quality, close to the highest in size (empty weight), above the average in capacity, considerably below the average in relation of capacity to weight, and slightly above the average in percentage of post-mortem recovery of milk.

Gross anatomy.—The cistern is below the average in relative size, and is divided and indistinct in outline. The tissue is dense and there is a very large quantity of connective tissue. The appearance of the udder may have been affected slightly by the fact that the udder was not well frozen when sectioned and as a result was somewhat torn by the saw. Udder 257, however, was in almost the same condition as regards completeness of freezing and showed a small quantity of connective tissue. After formalin treatment and sectioning the tissue was very wet, inclined to be sloppy, and lacking in firmness and spongy consistency.

Histology.—Sections from this udder showed only a limited amount of normal functionating glandular tissue. There had been some chronic irritation of the gland which resulted in a general increase in the connective tissue of the organ. The entire organ showed very marked degenerative and atrophic changes. Many of the alveoli showed detachment of the epithelium, and the lumen of a number were filled with the denuded epithelial cells and cellular débris. A great many milk calculi were observed. In places practically one-half of the acini were filled with these concretions. The area illustrated in Plate 10, was selected from a point near the center of the left rear quarter of the udder.

General.—When the udder was examined in February, 1926, the right front teat was distended with pus, and in March of the same year the same teat and quarter were described as hard. When the udder was examined before slaughter, the left rear quarter was slightly larger and hung lower than the others, but the udder was functioning in all quarters. The very abundant fiber noted on ante-mortem examination is in accord with the histological and gross-anatomy findings. The free space grading is also generally supported by the rating for size of cistern.

COW 443 (PRODUCTION GRADED AS MEDIUM TO LOW)

Cow 443, a registered Jersey, produced 9,621 pounds of milk and 465 pounds of butterfat under official testing conditions at the age of 6 years 4 months. Apparently this is indicative of her maximum producing ability. Her udder was about average in composite grade for quality, small in size (empty weight), low in capacity, close to average in relation of capacity to weight of udder, and next to the highest in the group in post-mortem recovery of milk.

Gross anatomy.—The cistern is proportionately very small and of the honeycomb rather than the distinctly circumscribed type. The gland tissue is very dense, free of visible ducts, and has unusually abundant connective tissue throughout. This udder, like Nos. 257 and 253, was not completely frozen when sectioned. The tissue after sectioning was very soft, wet, and lacking in firmness and in the usual spongy and rubbery consistency.

Histology.—In this udder sections from all the different areas in the front and rear quarters showed a number of abnormal changes.

The connective tissue framework of the gland had a soft, spongy appearance suggestive of an edematous condition of the organ. All the sections showed more or less detachment of the epithelial lining of the acini, and chronic inflammatory changes were also noted. In places whole groups of lobules had apparently been replaced by fat tissue. Many concretions varying in size were seen in the alveoli in the different sections. The area illustrated in Plate 11 was selected from a point near the base of the left front quarter.

General.—As far as records show, this udder had never experienced any noticeable disturbance. When examined before the cow was slaughtered, it was classed as in good condition. There were indications of large cisterns in all except the right rear quarter, which is the quarter used in illustrating the gross structure. (Pl. 11, A.) The histological findings can not be explained by any ante-mortem notations. The relatively advanced age of this cow should be considered in this connection, and the use of petroleum ether in the half subsequently used for histological study is noted.

COW 255 (PRODUCTION GRADED AS LOW)

Cow 255, a registered Holstein, produced 11,202 pounds of milk and 415 pounds of butterfat under official testing conditions at 2 years 8 months of age. In a later official test at 7 years 3 months she produced 11,480 pounds of milk and 392 pounds of butterfat. As her mature record did not exceed the record made at 2 years 8 months, the mature equivalent was not calculated. This cow undoubtedly represents the lowest producing capacity of any cow in the group. She was slaughtered one month after last freshening at the age of 9 years 7 months. Her udder was very slightly above the average in composite grade for quality, above the average in size (empty weight), well above the average in capacity, slightly below the average in relation of capacity to weight of udder, and about average in percentage of post-mortem recovery of milk.

Gross anatomy.—The cistern is of about medium size, very much divided into relatively large chambers, and indistinct in outline. The tissue is very open in structure and shows a large quantity of connective tissue. The tissue after treatment was very wet and inclined to be soft and mushy.

Histology.—This udder showed only one outstanding deviation from the normal, consisting of an infiltration of white blood cells into and between the alveoli of the gland. These cellular infiltrations, which were composed largely of eosinophile cells, were observed in all the sections examined. The fibrous framework of this gland was of very soft or loose texture. The area illustrated in Plate 12 was obtained from a point near the top of the left front quarter.

General.—The udder apparently was sound. It tilted forward slightly and the left side was somewhat heavier than the right. Records fail to show any disturbance requiring treatment of the udder at any time.

SUMMARY AND CONCLUSIONS

Separate grades for quality, based on ante-mortem examination of the udder, have been assigned to looseness of udder, yieldability of udder, softness and mellowness of gland tissue, abundance of fiber,

and average free space. A composite grade for all of these items is used in some parts of this study as a single indication of udder quality.

The size (empty weight) of the udders used in this study varied from 23.30 to 54.15 pounds and averaged 36.89 pounds.

The table of capacities shows that a cow's udder after amputation may be capable of holding as much as 6 gallons of milk. Six of the 11 udders had capacities of more than 50 pounds, and the average for the entire lot was 50.11 pounds. This indicates ample space for storing in the udder all the milk obtained at a milking. There is some probability, however, that the pressure resulting from the accumulation of milk in the udder gradually checks and finally stops milk secretion. If this is true, the most rapid secretion should take place soon after the udder is emptied, and the greatest total production should be obtained from cows milked often enough to keep the internal udder pressure sufficiently low to enable milk secretion to continue unchecked. This may explain why heavy producers yield greater quantities when milked three or four times than when milked only twice daily.

The relation of capacity to weight of udder is an index of the udder's fluid-holding capacity for each unit of empty weight, and should indicate its porosity. The percentages showing this relationship range from 100.98 to 243.10 and average 142.36. In other words, the capacity was greater than the weight in every instance, and on an average was nearly one and one-half times the weight of the empty udder.

It appears that a definite and positive correlation exists between udder quality and post-mortem recovery of milk after amputation of the udder. This is not only true for the composite grade for quality, but for all except one of the individual quality items, and indicates that milk can be drawn more readily from an amputated udder of good quality than from one of poor quality. Experiments are now in progress which should indicate whether or not high quality of udder is conducive to rapid milking in the living cow.

Udder quality and size (empty weight) of udder seem to be more or less negatively or inversely correlated. Every one of the correlations determined was negative. Those for yieldability of udder and composite grade for quality were significant, those for softness and mellowness of gland tissue and average free space were moderate in degree, and those for looseness of udder and abundance of fiber were not significant. On the other hand, a moderate positive correlation was shown to exist between size of udder and total yield of milk.

Apparently udder quality is not in any way associated with udder capacity so far as this group of udders is concerned.

Udder quality appears to be positively and rather definitely associated with relation of capacity to weight of udder. Every one of the correlations was positive. Those for yieldability of udder, average free space, and composite grade for quality were significant, that for softness and mellowness of gland tissue was very nearly significant, and those for looseness of udder and abundance of fiber were not significant.

A significant and negative or inverse correlation was found to exist between post-mortem recovery of milk and size (empty weight) of udder. This indicates that the milk was drawn more readily from the small udders than from the large ones. The small udder of good

quality seems to be the kind that yields its milk most readily on post-mortem milking.

Post-mortem recovery did not appear to be in any way associated with capacity of udder.

The post-mortem recovery was fairly definitely associated with relation of capacity to weight of udder. This suggests again a probable positive relationship between quality of udder and relation of capacity to weight of udder.

Apparently the post-mortem recovery of milk from amputated udders was not materially affected by the ante-mortem level of production at the time of slaughter.

Size (empty weight) of udder and capacity of udder do not appear to be definitely correlated.

The small udders had a greater capacity for each unit of empty weight than did the large ones.

Breed characteristics appear to have had little effect on the results shown in connection with udder quality, post-mortem recovery of milk, or size (empty weight) of udder but to have had some effect on the results based on udder capacity.

Stage of lactation in this group of cows does not seem to be associated with composite grade for quality, percentage of post-mortem recovery of milk, size (empty weight) of udder or capacity of udder. Apparently, therefore, the variation in stage of lactation was not of great importance as a disturbing factor.

Relative size of cistern (graded) appears to be positively and fairly definitely correlated with free space; its correlation with size (empty weight) of udder was distinctly negative or inverse; and its correlation with other udder characteristics was either moderate or not significant. The relative size of cistern (measured), which was particularly difficult to determine, was only moderately correlated with any of the udder characteristics studied in relation to it.

The relative openness or porosity of the gland tissue was not correlated with udder quality or with any of the other udder characteristics studied.

The relative abundance of connective tissue visible in the gross anatomy sections of the amputated udder, was positively and fairly definitely correlated with abundance of fiber which was graded ante-mortem, but does not appear to be correlated with any of the other udder characteristics studied.

The possibility of anticipating the gross structure of the udder by examining it ante-mortem appears from these results to be very limited.

The producing ability of the cows used in these studies as indicated by their milk records after adjustment for age and management, seems to be moderately correlated with size (empty weight) of udder, and distinctly correlated with udder capacity. The correlations between milk-producing ability and other ante-mortem and post-mortem udder characteristics were all very doubtful or of no significance. This consideration was based entirely on quantity of milk produced and made no allowance whatever for butterfat production or for breed characteristics.

Numerical ratings designed to give credit for the cow's efficiency as a producer by making allowance for the influence of breed charac-

teristics, were assigned to the production performance of each cow. These ratings were to a very large extent based on butterfat production. Correlation coefficients, determined to show the degree of relationship between these numerical grades and 14 different udder characteristics, were either of very doubtful significance or of no significance whatever. Little relationship was found to exist between ratings for production and udder characteristics.

The histological findings can in most cases be accounted for when consideration is given to the history of the udder, either through its stages of activity or its observed condition at and immediately before slaughter.

In most of these cases the period that elapsed between the completion of the production record given and the slaughtering of the cow, was long enough to provide ample time for the development of the abnormal conditions of the udder tissue reported in some of the histological examinations. It would be interesting to know how much the producing capacity of udders would be impaired by such abnormal developments in the mammary tissue. It seems desirable, therefore, to make histological examinations of the udder tissue of some high-producing cows soon after the completion of their production records.

The limitation in number of animals available for the study presented in this paper is fully appreciated. The results presented are more or less preliminary and are not conclusive. Additional studies of many of these considerations based on larger populations are under way.

RELATION OF THE SEMIPERMEABLE MEMBRANES OF THE WHEAT KERNEL TO INFECTION BY GIBBERELLA SAUBINETII[1]

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INTRODUCTION

Relatively little has been published on the formation of the so-called semipermeable membranes of the caryopsis of the Gramineae and their relation to kernel development and maturation. Likewise, the penetration of the cellulose and ligno-subero-cellulose cell walls and cuticularized epidermal surfaces of the kernel and the relation of these structures to invasion of the tissues by various microorganisms have not been studied in detail. Do such membranes in the cereal kernel play an important rôle in preventing aggressive invasion of the embryo and storage structures in the later stages of kernel infection? Are these membranes partly accountable for the presence of Fusarium and other organisms of this type in the outer structures of the kernel, as is so frequently found to be the case in lightly infected grain? Since not only the extent of the infection, but its location as well, largely determines the quality of the infected grain, it seemed especially important to study the kernel development in relation to the formation of these structures and their rôle in limiting infection by the wheat-scab parasite.

The results reported in this paper were obtained in connection with a general study of cereal Fusaria. The infected kernels studied were from naturally infected and artificially inoculated heads, fixed in 1923 and 1924 and worked up by the senior writer at that time. The infections were obtained on immature and mature kernels, the stage of development being determined by observation checked by later histological examination. The infected mature kernels were secured after a period of rainy weather from bundles of cut grain lying on moist ground where the fungus, either from natural infections or from artificial inoculations, had overrun the heads after the grain was ripe. Inoculations of mature wheat heads standing in the field or in the greenhouse made in connection with this study were unsuccessful, owing in all probability to the fact that no technic had then been perfected for supplying adequate moisture throughout the incubation period of the fungus. It has seemed advisable, however, to summarize the investigations and present the results in this paper.

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[2] The writers wish to express their thanks to Eugene H. Herrling, Department of Plant Pathology, University of Wisconsin, who made a large proportion of the photomicrographs used.

The difficulties usually experienced in cutting serial sections of mature kernels of the cereals were overcome by treating the killed and partially dehydrated grains with a mixture of equal parts of chemically pure hydrofluoric acid and 70 per cent alcohol for from 48 to 72 hours. Thorough washing in water for 24 hours, followed by the usual steps in the paraffin method of embedding, prepared the material for sectioning. Delafield's haematoxylin and erythrosin following Juel's zinc chloride fixative [3] gave a color contrast between host and fungus. Acidified Sudan III [4] was used to demonstrate the presence of suberized or cutinized parts.

HISTORICAL REVIEW

General interest in these semipermeable layers of the kernel was awakened by Brown (*2*)[5] in 1907 in his work with barley. He used *Hordeum vulgare* var. *coerulescens*, which has a blue pigment in the aleurone cells that is turned red by acid, thus furnishing a natural indicator for the study of permeability in relation to acids. In his conclusions he states (1) that the grain of Hordeum is inclosed within a semipermeable or selective covering; (2) that the semipermeable property of the covering of the grain of Hordeum is not due to the action of living protoplasm; (3) that the semipermeable property of the covering of the grain of Hordeum is located in the spermoderm of the grain; and (4) that the grain of Avena, Triticum, and Secale is inclosed in a semipermeable covering apparently similar to that of the grain of Hordeum.

The exact location of the semipermeable property of the covering was not known for some time. In publishing on later researches, Brown (*3*, *p. 84*), in 1909, says:

> Histological study of the seed-coverings indicates that their selective power is confined to the testa, and probably to that portion which is derived from the epidermis of the nucellus during the development of the seed.

Schroeder (*8*), working with wheat in 1911, was of the opinion that penetration by liquids was not uniform over the surface of the grain. He thought that entrance was effected chiefly at the embryo end or that there was increasing impermeability of the coverings from the basal toward the apical end of the grain. Eckerson (*5*), working with wheat in 1917, spoke of the outer membrane of the nucellus as a semipermeable membrane.

Collins (*4*), working with barley in 1918, was the first to study seriously the structure of the integumentary system in relation to localized water absorption and semipermeability. He steeped the grains in water for two days and then cut transverse sections through the mid region by hand. Concerning structure in the region of the furrow he says (*4*, *p. 384–385*):

> The furrow corresponds in position and extent with an elongated chalazal tract, through which nutriment and reserve materials pass from the vascular supply in the ovary wall to the cells of the endosperm. The tissues of the pericarp and ovule are continuous; indeed this elongated tract is to be regarded as the base of the ovule—the extended chalaza from the flanks of which the integu-

[3] Juel's fixative: Zinc chloride, 10 g; acetic acid, 10 g; 50 per cent alcohol, 500 c c.

[4] Acidified Sudan III: Saturated solution of Sudan III in 70 per cent alcohol; 8 to 10 drops of H_2SO_4 per 100 c c of solution.

[5] Reference is made by number (italic) to Literature Cited, p. 625.

ments originate. On the ovular side of the vascular bundle, and lying immediately between the points of origin of the tegmen, is a group of cells of glandular character with somewhat thickened walls and yellowish homogeneous contents. In the developing grain the contents appear to be of an oily nature. Radiating from this group, towards the centre of the developing grain, is a sheaf like mass of elongated cells which serve to distribute supplies to the endosperm.

The epidermis of the nucellus extends, he says, immediately within the tegmen, up to its origin, but not across the chalazal tract itself. As the nucellar epidermis does not delimit the cells of sheaflike structure, it can not obstruct the passage of salts at this point.

Concerning the extent of the outer membrane of the tegmen, his sections of immature ovules showed that it completely enveloped the ovule except at the micropyle and in the chalazal tract. In longitudinal sections of the mature grain the micropylar point was not satisfactorily made out, although the membranes could be traced up to a dark-colored caplike covering to which was attached the embryonic appendage. Concerning the embryonic appendage he says (*4, p. 385*):

> This is a group of cells forming the apex of the root-sheath, which, lying immediately beneath the micropyle, makes real contact between the embryo and the tegmen. The cells differ from those of the root-sheath proper in that they have no contents and are capable of very rapid swelling when placed in contact with water. Although the actual origin of these cells has not been proved yet, the author is of the opinion that they may represent a suspensorial group.

He says that real contact between tegmen and membrane is also made "over the peripheral rim of the scutellum through the agency of a single layer of modified aleurone cells." He concludes in relation to the entrance of water—

> that the micropyle is the point of rapid entry and the seat of differential action through the agency of the embryonic appendage, whilst slow, differential filtration takes place across the chalazal tissue lying along the furrow,

movement of liquids being most rapid at the point "where it meets the dorsal margin of the scutellum."

Braun (*1*), in 1924, found a gradient of permeability to iodine in the wheat seed coats. He says the entrance of iodine takes place over the entire surface of the grain, radially, but not uniformly. The apparent lateral and distal spread from the embryo end is due to the existence of a gradient of permeability to iodine in the seed coats, which permeability is greatest near the embryo end, diminishes to a minimum near the distal end, and again increases slightly at the distal apex. Microtome sections of immature wheat seeds fixed in Flemming's strong solution showed a deep blackening of the cutin layer near the distal end with scarcely any discoloration at the embryo end and a gradient of discoloration between. In mature seeds the cutinization gradient was found to be nearly the same as that determined by the use of Flemming's triple stain.

The chemical nature of the semipermeable membrane or membranes has been variously described. Eckerson (*5*) says the semipermeable membrane of wheat is suberized. Collins (*4*) found that the tegmen membranes of barley grains were cuticularized at an early stage of development. Gordon (*6*), in her study of the development of the endosperm in cereals, calls the testa of barley a layer impregnated with silica, and Braun (*1*) speaks of a cutin layer in connection with his studies of permeability to iodine in the wheat seed coats.

A report of a study on the anatomy of wheat kernels infected with a Fusarium, made in 1889 by Woronin, is found in a publication by Palchevsky (*7*) which appeared in 1891. Woronin carried on investigations with wheat infected with a Fusarium which he called *F. roseum* Link and which he considered to be the conidial stage of *Gibberella saubinetii*. He notes that sometimes infection is localized only in the embryo and on adjacent parts while the rest of thek ernel remains normal. Concerning the anatomy of the kernel infected before maturity he states:

> The anatomical picture of the wheat kernel is quite different from that of the normal grain. The mycelium, which is often of an intense purple color, penetrates from the periphery of the kernel through the seed coat and the aleurone layer into the endosperm of the seed, spreading in all directions. The shrunken periphery of the kernel, seen through a microscope, is wavy, and the epidermis of the ovary sometimes remains green (chlorophyll), for the fungus attacks kernels that are not ripe.
>
> The pigment layer of the kernel, slightly darker than normal, forms accumulations here and there. Between the pigment and the aleurone layer, that is, in the place of the layer of thick-walled cells, there is a dense mycelium which often surpasses the seed coat in thickness and occasionally forms tumors.
>
> The aleurone layer is wavy, interrupted, and has the appearance of a dark stripe; there are many dark spots in its contents, and in general it is darker than normal; however, it stains normal crimson with SO_3 (A. S. purum) with sugar sirup. In such grains containing mycelium the cell structure, especially of the seed-coat layers, is destroyed. The starch grains are connected by fungus threads, like clusters of grapes; the smaller grains seem to be firmly attached to them. [6]

STRUCTURE OF KERNELS

MORPHOLOGICAL ORIGIN OF THE TESTA

The testa is derived from the inner integument of the ovule. In the very young grain, both the inner and the outer integuments consist of two layers of cells. (Fig. 1, A.) The outer integument disintegrates and disappears very early in the development of the grain. The inner integument changes more slowly (fig. 1, B) and persists in a modified form. Before the developing kernel occupies the entire nucellar space, sections stained with Sudan III show very thin red lines, membranes of equal thickness on both borders of the inner integument. (Fig. 2, A.) The membrane on the outer wall thickens rapidly (fig. 2, C); the inner one remains so thin that in mature kernels it is likely to be overlooked. These membranes are unaffected by the ordinary cytological stains. Their reaction to Sudan III and their solubility in sulphuric acid indicate the presence of suberinlike or cutinlike compounds in the walls and on the outer surface.

As development proceeds, the outer cells of the integument lose most of their contents and collapse. At the same time the contents of the inner row collect in masses which seem to contain globules at first and which later have a homogeneous waxy or oily appearance. (Fig. 1, B; fig. 2, C.) The masses take on color as they collect, varying from golden to golden brown. The walls of these cells also become brownish. As the kernel matures the remains of the two rows of cells of the inner integument are crushed into a continuous narrow golden-brown layer commonly called the color layer of the wheat

[6] Translation by I. V. Krasovsky

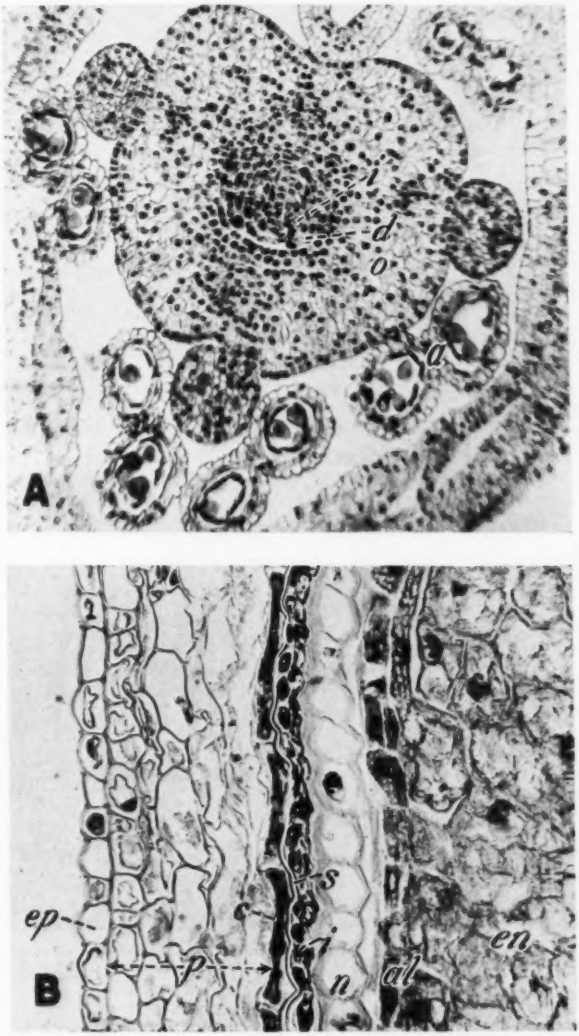


FIGURE 1.—A, Cross section of a portion of a wheat flower: *a*, Anther; *o*, ovary; *d*, outer integument; *i*, inner integument. Stained with Flemming's triple stain. × 130. B, Cross section showing the respective layers of an immature wheat kernel: *ep*, outer epidermis of pericarp; *p*, pericarp; *c*, chlorophyll (cross) layer; *s*, outer membrane of the testa; *i*, cells of inner integument; *n*, epidermis of nucellus; *al*, aleurone cells forming; *en*, endosperm. Stained with Delafield's haematoxylin and erythrosin. × 300

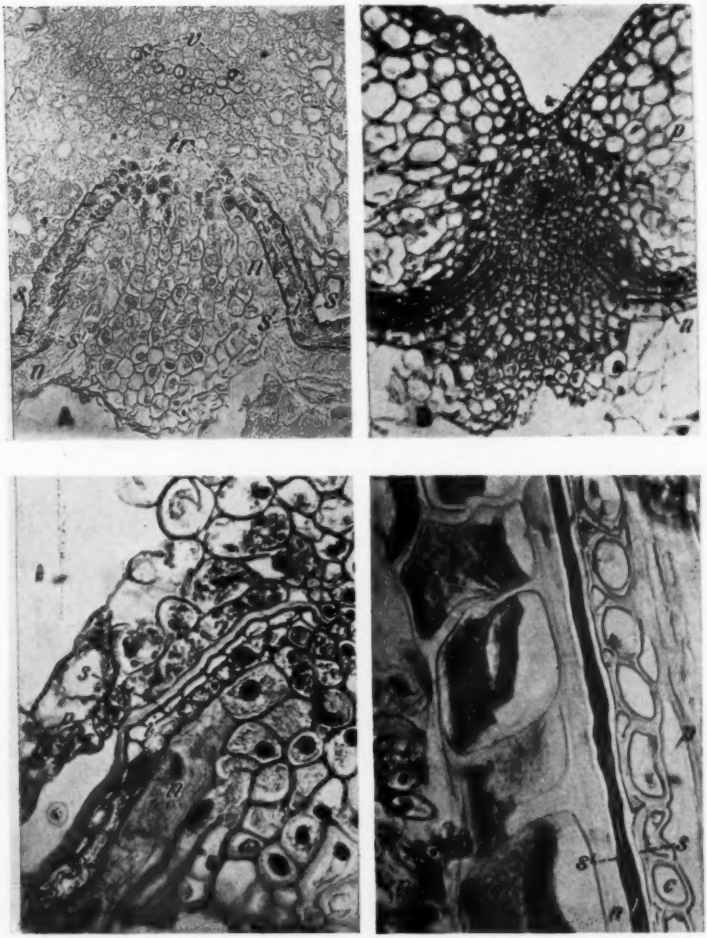


FIGURE 2.—A, Cross section through the groove region of a young kernel, showing the points of origin of the testa, the two rows of cells of the integument (*i*) bordered by membranes of equal thickness, *s* and *s'*; *n*, epidermis of nucellus; *v*, vascular trace of groove; *tr*, transchalazal area. Stained with Sudan III. × 178. B, Cross section of the transchalazal region in the groove of an immature kernel, showing the location of the points of origin of the inner integument and the epidermis of the nucellus; *p*, pericarp; *i*, inner integument; *n*, epidermis of nucellus. Stained with Delafield's haematoxylin and erythrosin. × 104. C, Cross section of a portion of an immature kernel, showing the thick membrane along the outer wall of the outer row of cells of the inner integument near its point of origin in the groove; *s*, outer membrane of testa; *i*, cells of inner integument; *n*, epidermis of nucellus; *p*, pericarp. Stained with Flemming's triple stain. × 328. D, Longitudinal section showing width and structure of the several layers in a mature wheat kernel; *s*, outer membrane of testa; *i*, color layer of testa; *s'*, inner membrane of testa; *n*, epidermis of nucellus; *al*, aleurone cells; *en*, endosperm; *c*, chlorophyll cells, cross layer, of pericarp; *p*, parenchyma of pericarp. Stained with Delafield's haematoxylin and erythrosin. × 535

grain. (Fig. 2, D.) The compression process in the filling of the kernel is completed when the grain is mature and is completed last at the basal end of the seed and in the groove on either side of the chalazal region. This layer together with the membranes bordering it on either side constitutes the testa of the mature kernel.

EXTENT OF THE TESTA

As noted previously, the testa of the mature grain is derived from the inner integument of the ovule (fig. 2, B) and is coextensive with it. Both cross (fig. 3, C) and longitudinal (fig. 3, B) sections of kernels show that it envelops the seed except at the micropyle and in the extended chalazal region of the groove (fig. 3, A). Cross sections of a kernel midway between the embryo and the brush end show that it originates sharply at either side of the vascular trace of the groove. (Fig. 4, A.)

Along the groove between the lines of origin of the testa are cells which have some of the characters of the cells of the inner integument, but whose exact nature was not determined. Collins speaks of them in barley as "cells of glandular character." The contents of these cells appear to pass through the changes described for the contents of the cells of the inner integument in the formation of the color layer, becoming oily or waxy in appearance and golden brown in color. The changes first appear in scattered cells, beginning shortly after the corresponding changes in the inner integument. They seem to progress less rapidly and are completed last at the base of the chalazal region. (Fig. 5, A and C.) When the kernels are mature, the transchalazal area is filled with a compact group of cells similar to the color layer in the appearance of the cell contents. The walls of these cells are thicker than the adjacent parenchyma of the pericarp, and stain a deep red with safranine (fig. 5, A), as do the cell walls in the color layer (fig. 2, C). When treated with acidified Sudan III, they stain a light red not observed in the compressed color layer, indicating the presence of suberin or cutin. These membranes, like the layers of the testa, are quite resistant to the action of 72 per cent H_2SO_4.

Cells whose contents appear to be similar to those of the transchalazal area are found bordering the micropyle, but here they are clearly a part of the color layer. A cross section through the micropyle indicates that the color layer folds outward and back at the opening and is connected at its end with the end of the outer layer of the testa. (Fig. 5, D.) In the vicinity of the micropyle the testa does not undergo compression as is the case in the regions bordering the endosperm, and hence the color layer in this region does not lose its appearance of cellular structure. (Fig. 5, D; fig. 4, C.) Collins states that the walls bounding the micropyle were found to be cuticularized. It was not clear from the sections examined whether the thin inner membrane follows the surface of the color layer as it folds outward at the micropyle, but probably it does.

RELATIVE THICKNESS OF THE OUTER LAYER OF THE TESTA

The outer layer of the testa of the wheat kernel is not of uniform thickness throughout its extent. (Fig. 6, A and C.) The thickest portions are found in the groove, along the top of the kernel where it

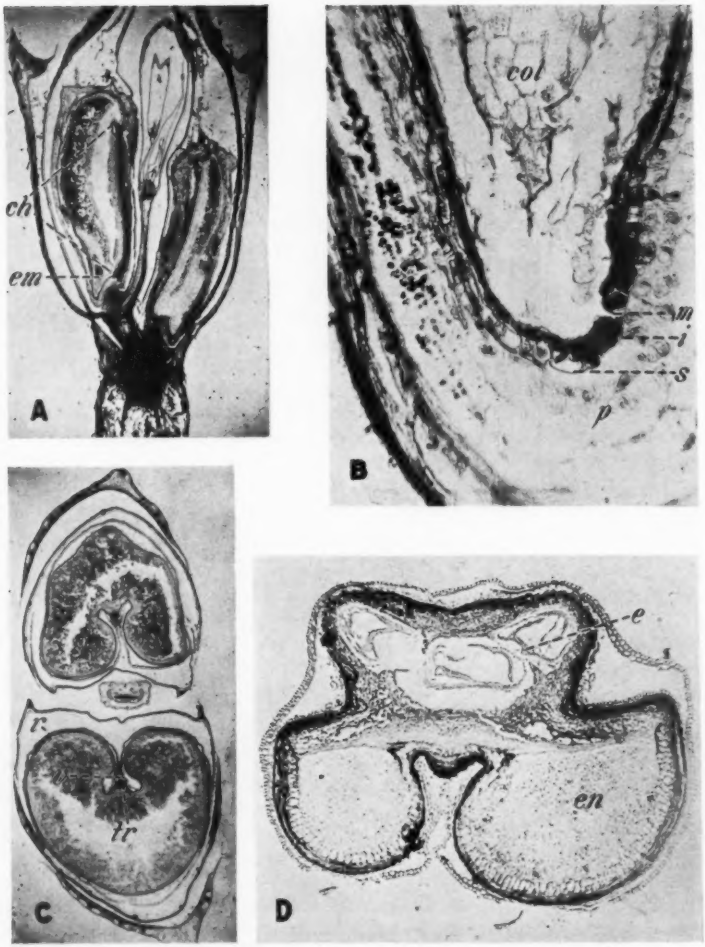


FIGURE 3.—A, Longitudinal section of a spikelet containing two kernels, showing the location and extent of the chalazal region (*ch*) and the position of the embryo (*em*). Stained with Flemming's triple stain. × 9. B, Longitudinal section through the radicle of an immature kernel, showing the opening through the testa at the micropyle; *s*, outer membrane; *i*, cells of inner integument; *m*, opening of the micropyle; *col*, coleorhiza; *p*, pericarp. Stained with Flemming's triple stain. × 220. C, Cross section of a spikelet containing two kernels, showing the location of the testa (*r*), transchalazal group of cells (*tr*), and the vascular trace of the groove (*v*). Stained with Flemming's triple stain. × 8. D, Cross sections of an infected kernel, showing the resulting destruction of the embryo; *e*, embryo; *en*, endosperm. Stained with Delafield's haematoxylin and erythrosin. × 22

FIGURE 4.—A, Cross section through the groove showing the points of origin of the testa (*s* and *i*), also the scattered cells in the transcha'azal region (*tr*) which have taken on a golden-brown color similar to that of the color layer (*i*); *v*, vascular trace of the groove. × 136. B, Portion of a longitudinal section through the embryo of a wheat kernel, sowing the relatively thick outer membrane (*s*) of the testa at the base of the chalazal region (*ch*); *s'*, inner membrane of testa. The cells of the inner integument (*i*) do not undergo severe compression at this point. Stained with Sudan III. × 205. C, Longitudinal section through the micropyle (*m*) of a mature wheat kernel; *s*, outer membrane of testa; *i*, color layer (inner integument). Stained with Sudan III. × 375

passes under the style (fig. 5, B), and in the region extending from the micropyle to the base of the groove (fig. 4, B). It is thinnest over the embryo. Serial sections show that its thickness is fairly uniform in the groove from the basal to the brush end of the kernel.

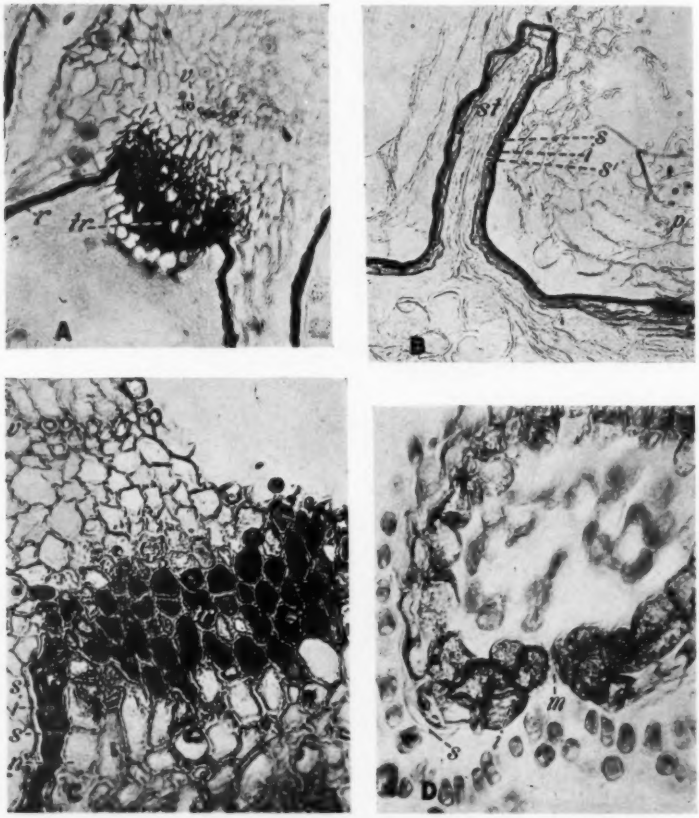


FIGURE 5.—A, Cross section through the groove, above the embryo, of a fairly mature wheat kernel, showing the testa (*r*) and the transchalazal group of cells (*tr*); the walls of both color layer and transchalazal cells and much of the cell contents stain red with safranine; *v*, vascular trace of the groove. Stained with Flemming's triple stain. × 75. B, Portion of a longitudinal section of a kernel, showing the thickness of the outer membrane of the testa under the remains of the style (*st*); *s*, outer membrane; *i*, color layer of testa; *s'*, inner membrane of testa; *p*, pericarp. Stained with Sudan III. × 173. C, Cross section through a portion of the chalazal region at the embryo end of the kernel, showing the slightly thickened walls and the deeply stained cell contents of the transchalazal group (*tr*); *s*, outer membrane of testa; *i*, color layer; *s'*, inner membrane of testa; *n*, epidermis of nucellus; *v*, vascular trace of groove. Stained with Flemming's triple stain. The walls of the transchalazal cells in this region do not stain as deeply with safranine as do those midway of the same kernel, shown in A. × 317. D, Cross section through the opening of the micropyle (*m*) of a mature wheat kernel; *i*, color layer; *s*, outer membrane of testa. Stained with Delafield's haematoxylin and erythrosin. × 288

On the dorsal side, where the membrane is thin, there is a gradual but slight increase in thickness up to the brush end, where a more abrupt increase is found.

EXTENT OF THE EPIDERMIS OF THE NUCELLUS

In immature kernels the epidermis of the nucellus is seen as a row of cells lying immediately beneath the inner integument of the ovule and may be traced to its lines of origin along the groove. (Fig. 2, A,

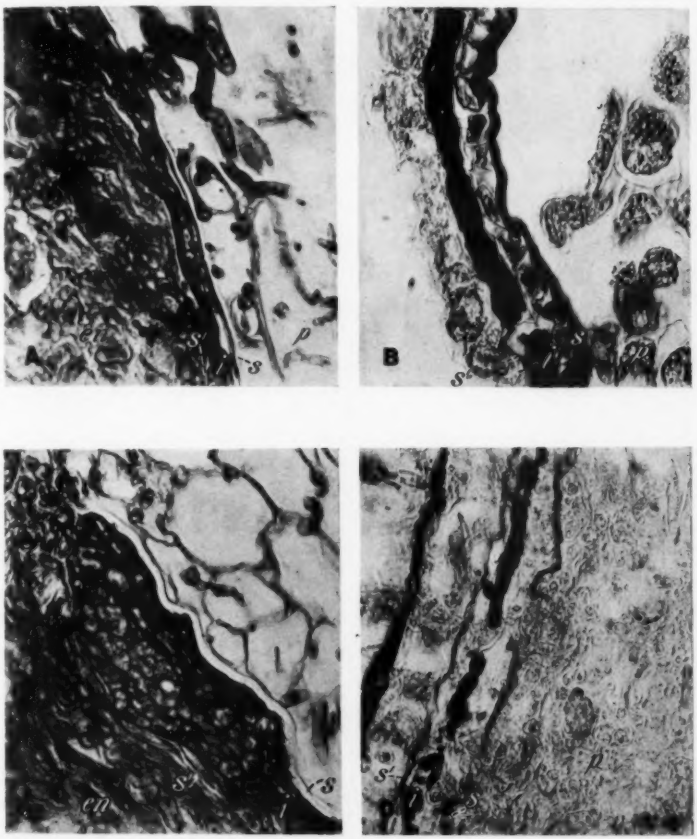


FIGURE 6.—A and C, Cross sections showing the relative thickness of the outer membrane of the testa over the embryo of the mature wheat kernel (A) and in the groove region (C) of the same section of an infected kernel. In C hyphae are so massed in the testa that the two membranes *s* and *s'* are widely separated; *i*, color layer; *en*, endosperm; *p*, pericarp. Stained with Delafield's haematoxylin and erythrosin. × 385. B. Portion of a longitudinal section through the immature and uninfected second kernel of a spikelet. × 467. D, Section through the same region in the infected basal kernel of the spikelet. Comparison of the thickness of the outer membrane of the testa in the two kernels indicates that infection of the basal kernel occurred at a considerably earlier stage of development than that pictured in kernel B. Stained with Sudan III and gentian violet. × 350

B, and C.) In mature kernels it is compressed and appears as a band without visible cellular structure. (Fig. 2, D.) According to Collins (*4*), the epidermis of the nucellus in barley does not cross the chalazal region. The same is true of wheat. However, in some sections of mature kernels a band similar in appearance but irregular in

width may be found bridging the groove at some distance from the ends of the testa and connecting with the epidermis of the nucellus on either side. Apparently this irregular band is the compressed remains of nucellar tissue which did not disintegrate during the process of maturing.

RESISTANCE TO INFECTION

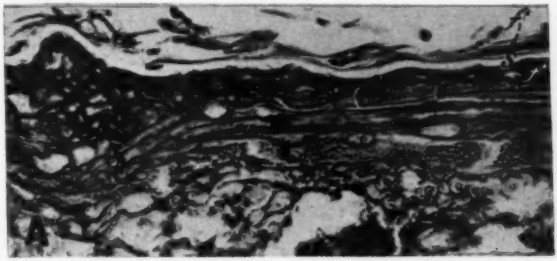
In correlating the tissues of the wheat kernel with infection it may be said, in general, that the testa becomes increasingly resistant to penetration by *Gibberella saubinetii* as the grain matures. In cases of early infection the membranes may be penetrated in numerous places, as shown in Figure 6, D. Figure 6, B, pictures a location similar to that of Figure 6, D, in the uninfected younger second kernel of the same spikelet. The comparison of Figure 6, B and D, indicates that infection of kernel B occurred at an early stage in its development. The cellular structure of the color layer in B shows the second kernel to be immature, and the greater thickness of the outer membranes of the testa indicates that infection in the older basal kernel, D, must have occurred at a considerably earlier stage of development than that now shown in kernel B.

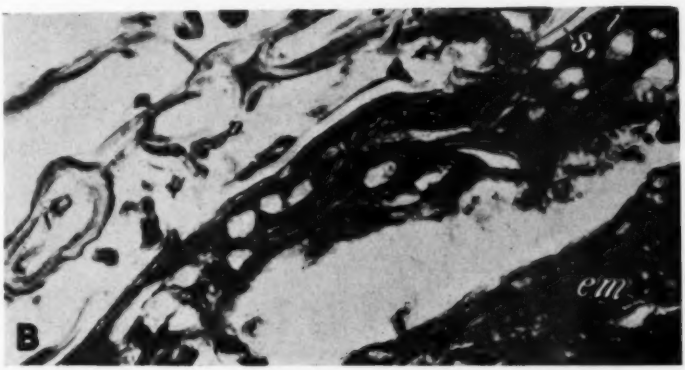
The outer layer of the testa, the semipermeable membrane, is the part of the kernel most resistant to penetration. It may often be followed in parts of kernels so badly infected that other tissues can scarcely be identified except by their position (fig. 7, C), and it is not unusual to find it intact for considerable distances with mycelium in contact with one or both surfaces (fig. 7, A). Numerous sections show the fungus permeating the color layer and at times becoming so thickly massed there as to force the two membranes of the testa widely apart. (Fig. 6, C.) In such cases it seems probable that the hyphae had entered this layer before it became compressed into a narrow continuous band. In the early stages of development there is no visible barrier to entrance into this layer at its point of origin in the groove region. (Fig. 2, A and B.)

After the outer membrane of the testa has become thickened sufficiently to offer some resistance to the entrance of the fungus there is a period in which the young wheat kernel seems to be relatively unprotected in the groove region. (Fig. 2, A.) As development proceeds, the group of cells in the chalazal region takes on color (fig. 4, A) and the walls thicken slightly and fill the gap between the points of origin of the testa. It does not appear that in the immature kernel this area offers as effective resistance to hyphal penetration as does the outer layer of the testa; nevertheless it serves as a barrier of marked efficiency during the later stages of development of the kernel. (Fig. 5, A.)

The degree of resistance of the membranes of the testa is seemingly proportional to their thickness. Hence, penetration of the outer membrane occurs more often in the region over the embryo than elsewhere. (Fig. 6, A and D.) Though the outer membrane of the testa is the most resistant part within the kernel, it is not impenetrable in any location, and in cases of severe infection hyphae may pass through even the comparatively thick portions. (Fig. 7, B.)

The compressed nucellus shows little more invasion than the testa, owing probably not so much to resistance as to the fact that its thick-





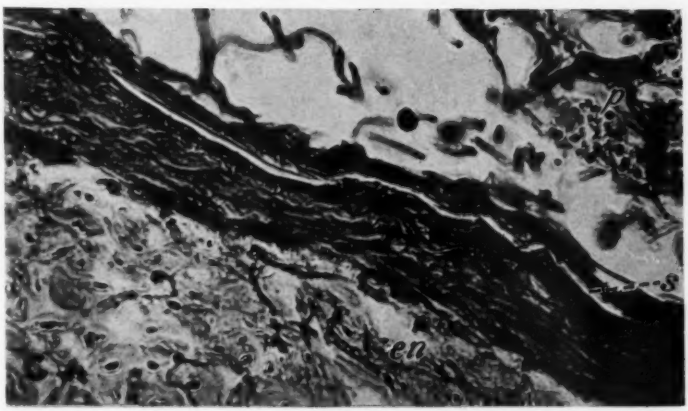


FIGURE 7.—A, Portion of a cross section in the groove of an infected kernel. The outer membrane of the testa (*s*) is intact for considerable distances, though fungus hyphae (*f*) are in contact with both surfaces. Stained with Delafield's haematoxylin and erythrosin. × 446. B, Portion of a longitudinal section of a wheat kernel containing a badly infected embryo; this section is at the flank margin of the embryo, where the outer membrane of the testa is comparatively thick; hyphae have penetrated the membrane in numerous places; *em*, embryo; *i*, color layer of testa; *s*, outer membrane of testa; *p*, pericarp. Stained with Delafield's haematoxylin and erythrosin. × 670. C, Portion of a cross section of a badly infected immature wheat kernel; the outer membrane of the testa (*s*) is visible for considerable distances; the color layer, inner membrane of the testa, epidermis of the nucellus, and aleurone cells are indistinguishable in the mass of hyphae; *en*, endosperm; *p*, pericarp. Stained with Delafield's haematoxylin and erythrosin. × 446

ness and chemical composition make it less attractive to the fungus and hence somewhat of a barrier to hyphal advance.

The scarcity of fungus in the endosperm probably is explained by the lack of sufficient moisture to make growth possible and to the utilization by the fungus of pentosan materials more readily than hexose compounds rather than to any resistance in that area.

DISTRIBUTION OF THE FUNGUS WITHIN THE KERNELS

The fungus penetration of the wheat kernel at the brush end appears to be accomplished with little difficulty at flowering time, when hyphae advance from infected anthers to the ovary. The fungus may permeate the tissue of the kernel quite thoroughly, for the hyphae travel rapidly along the intercellular spaces, and the thin-walled cells of the parenchyma offer little resistance to penetration. At that immature stage the membranes of the testa are thin and the transchalazal group of cells shows little specialization, which leaves the young seed poorly protected. Under such conditions it is usual to find the fungus advancing from the pericarp into the young kernel. The result is that when infections occur at flowering time a comparatively high percentage of kernels are so badly diseased as to be practically worthless.

The distribution of the fungus in kernels infected after they have reached maturity is generally as follows: The pericarp with a light infection throughout or at least at the embryo end; the testa comparatively clean, with the possible exception of penetrations in the embryo region where the outer membrane is thin; the epidermis of the nucellus comparatively clean though showing more infection than the testa; hyphae in some cases massed between the various layers, separating them more or less widely (fig. 8, A and B); the aleurone cells filled with fungus (fig. 8, A) in the embryo region and for a considerable distance toward the brush end in the groove, a shorter distance on the dorsal side, and a much shorter distance on the flanks; the endosperm slightly infected near the embryo; and the embryo almost completely destroyed by the fungus (fig. 3, D.)

It is significant that late infections are more or less localized in the embryo end of the grain, where the outer layer of the testa remains thin and the color layer has not undergone severe compression, and where there is also the opening at the micropyle. Furthermore, it seems possible that the entrance of mycelium at the base of the chalazal region (fig. 3, A; fig. 4, B) might result in the prior infection of the embryo region. One such case was observed. In most cases, however, the embryos were so badly infected that it was impossible to discover the point at which the fungus had first entered the tissues.

Cases were observed in which, at various points along the groove, mycelium was apparently making its way from the pericarp through the irregularly placed cells which stain like the color layer. Perhaps this explains the common infection of the aleurone cells toward the brush end in the groove rather than at other points.

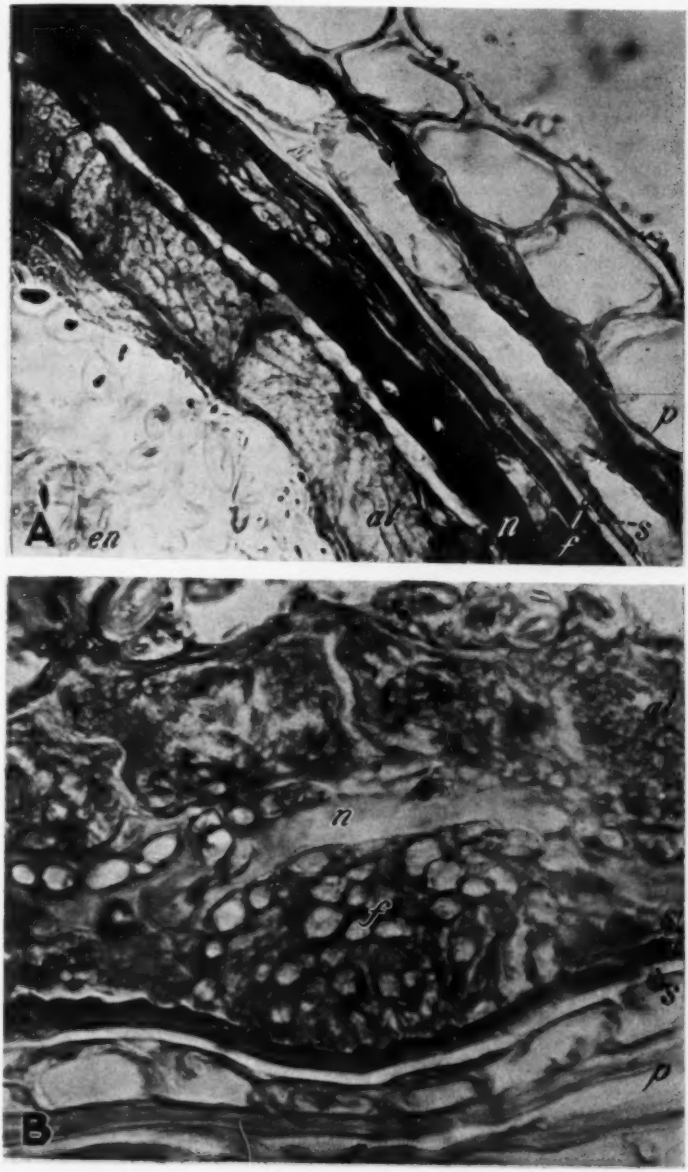


FIGURE 8.—A, Section of an infected wheat kernel, showing hyphae (*f*) massed in the aleurone cells (*al*) on either side of the nucellar layer (*n*) and penetrating the latter in a few places; *en*, endosperm; *p*, pericarp; *s*, outer membrane of testa; *i*, color layer of testa. Stained with Delafield's haematoxylin and erythrosin. × 600. B, Portion of a longitudinal section of a wheat kernel, showing fungus massed between the testa and epidermis of the nucellus, separating them widely in places; *n*, epidermis of nucellus; *s'*, inner membrane of testa; *i*, color layer of testa; *s*, outer membrane of testa; *al*, aleurone cells; *f*, hyphae; *p*, pericarp. Stained with Delafield's haematoxylin and erythrosin. × 940

DISCUSSION

The distribution of fungus in the wheat kernels infected after maturity is somewhat like the advance of "blueing" described by Collins (*4, p. 400*). He used iodine in potassium iodide and two varieties of barley. He states:

> In both cases the first signs of blueing of the starch contents appeared on each side of the furrow at the germ or basal end. From these points it was easy to trace the path taken by the iodine. Whilst it spread to some extent along each side of the furrow toward the apex of the grain, it passed more rapidly around the starchy endosperm rim immediately beneath the scutellum. The two blue arcs joined around the embryo, completing the circuit; at the same time the blueing spread rapidly on the curved side of the grain towards the apex, the lateral edge of colour as a rule being somewhat sharply delimited. The flanks of the furrow were the last to be blued.

The sections used in the present study showed numerous instances in which the forming color layer of the testa was permeated by hyphae. Concerning the passage of solutes through this layer Collins says (*4, p. 411–412*):

> Careful consideration has been given to the possible passage of solutions along the cells of the tegmen, between its inner and outer cuticularized membranes, and the selective action of the inner. The inner cuticle is less prominent and resistant, and might fail after a time. The embryonic appendage might serve as a temporary hindrance to the passage of solutes into the embryo, whilst the main stream would pass between the cuticularized membranes and be distributed in the distal direction. It should be said here that such a method of distribution assumes that the connecting anticlinal walls between the inner and outer membranes at the micropyle fail to resist the passage of solutes. In the ovule these walls bounding the micropyle were found to be cuticularized.

In speaking of the mature kernel he says (*4, p. 413*):

> The initial uptake of water supplies the need of the embryo. * * * The subsequent distribution of liquid in the endosperm is precisely the path of enzyme disintegration within the endosperm during germination of the embryo.

From the evidence at hand it seems probable that several factors influence the distribution of fungus hyphae within the kernel, i. e., the relative resistance of different parts to fungal penetration, the location of readily available food, and the distribution of water within the kernel.

In the very young wheat kernel, hyphae may be found in all parts. At this stage the membranes and cells are not sufficiently developed to offer any great resistance to the advance of the fungus; likewise, available food and moisture are plentiful. Gibberella has the ability to utilize a variety of food substances, especially pentosans; hence, the distribution of the abundant hexose nutrients within the kernel probably is only a minor factor influencing the location of the fungus. On the other hand, the location and structure of the protective parts, especially the layers of the testa, and the distribution of moisture within the grain appear to be important factors influencing the entrance and spread of *Gibberella saubinetii* within the wheat kernels.

SUMMARY

The testa of the mature wheat kernel is derived from the inner integument of the ovule and is coextensive with it. At maturity it consists of a narrow, homogeneous, golden-brown, oily-appearing layer formed by the compression of the two rows of degenerating cells of the inner integument and bordered on the inner side by a very thin color-

less membrane and on the outer side by one of considerable thickness. Both membranes stain with Sudan III and are relatively resistant to sulphuric acid, indicating the presence of suberinlike or cutinlike substances.

The testa completely envelops the seed except at the micropyle and in the groove region, where it is seen to originate sharply at either side of the vascular trace of the groove. In the chalazal region between the lines of origin of the testa there is a group of cells which have some of the characters of the cells of the inner integument but whose exact nature was not determined.

The outer layer of the testa varies in thickness. The thickest portions are found in the groove, along the top of the kernel where it passes under the style, and in the region extending from the micropyle to the base of the groove. It is thinnest over the embryo. On the dorsal side there is a gradual but slight increase in thickness up to the brush end.

In mature kernels the epidermis of the nucellus is compressed into a band without visible cellular structure. It does not cross the groove of the kernel.

The testa becomes increasingly resistant to penetration by *Gibberella saubinetii* as the grain matures. The degree of resistance of the membranes seemingly is proportional to their thickness. The outer membrane of the testa is the most resistant layer of the kernel.

Kernels infected at flowering time may be permeated throughout by the fungus.

In wheat grains infected at maturity the fungus is usually localized at the embryo end of the kernel; it is sparse in the testa, nucellar layer, and endosperm; the aleurone cells may be filled with hyphae for considerable distances along the groove, for a shorter distance on the dorsal side, and a considerably shorter distance on the flanks; the embryo is more or less completely permeated by fungus hyphae.

The location and structure of the protective parts, especially the layers of the testa, and the distribution of water within the kernel appear to be important factors influencing the entrance and spread of *Gibberella saubinetii* within the wheat kernel.

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A THREE-YEAR STUDY OF THE CHEMICAL COMPOSITION OF GRASS FROM PLOTS FERTILIZED AND GRAZED INTENSIVELY[1]

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INTRODUCTION

This paper is based on results obtained in 1928, 1929, and 1930 from an extensive trial of the so-called Hohenheim system of pasture management on the Massachusetts State College farm. Two papers [5] have been published giving the results for 1928 and 1929, respectively. This paper combines the results for all three seasons. The variations in the schedule of fertilizer application in the different years are shown in detail in Table 1.

TABLE 1.—*Schedule of fertilizer applications used each year, 1928, 1929, and 1930*

Year, plot group, and treatment	Elements applied	Pounds per acre applied	Dates of application
1928			
A, Completely fertilized; grazed throughout the season.	Nitrogen (N)[a]	85	Apr. 20–May 5.
	Phosphoric acid (P_2O_5)	55	
	Potash (K_2O)	67	
	Nitrogen (N) [b]	10	At intervals throughout the season, varying from 30 to 58 days, commencing May 28, ending Sept. 6.
	do	10	
B, completely fertilized but cut for hay in June; grazed from July 1.	Nitrogen (N)	85	May 7 and 8.
	Phosphoric acid (P_2O_5)	55	
	Potash (K_2O)	67	
	Nitrogen (N)	10	At intervals throughout the season, varying from 20 to 66 days: commencing June 21, ending Sept. 15.
	do	10	
	do	10	
D, control plot	No fertilizer		
1929			
A and B	Nitrogen (N)	41	Apr. 20–May 2.
	Phosphoric acid (P_2O_5)	41	
	Potash (K_2O)	50	
	Nitrogen (N)	15	At intervals throughout the season, varying from 26 to 56 days; commencing May 28, ending Aug. 29.
	do	15	
	do	15	

[a] The complete fertilizer used in all three years contained 16.5 per cent N, 16.5 per cent P_2O_5, and 20 per cent K_2O in 1928 and 1929. In 1930 the potash had been increased to 21.5 per cent.

[b] The source of nitrogen for summer applications in 1928 and 1929 contained 34 per cent N, and 13 per cent lime (CaO).

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[3] Appointed Jan. 2, 1930.

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[5] ARCHIBALD, J. G., and NELSON, P. R. THE CHEMICAL COMPOSITION OF GRASS FROM PLOTS FERTILIZED AND GRAZED INTENSIVELY. Jour. Amer. Soc. Agron. 21: 686–699, illus. 1929.

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TABLE 1.—*Schedule of fertilizer applications used each year, 1928, 1929, and 1930*—Continued

Year, plot group, and treatment	Elements applied	Pounds per acre applied	Dates of application
1929—Continued			
C, no nitrogen; grazed throughout the season.	Phosphoric acid (P_2O_5) [c]	41	May 2.
	Potash (K_2O) [d]	50	Do.
D, control plot	No fertilizer		
1930			
A and B	Nitrogen (N)	33	Apr. 3–15.
	Phosphoric acid (P_2O_5)	33	
	Potash (K_2O)	43	
	Nitrogen (N) [e]	30	May 29–July 9.
	Nitrogen (N)	20	July 25–Sept. 5.
C	Phosphoric acid (P_2O_5)	33	Apr. 15.
	Potash (K_2O)	43	Do.
D, control plot	No fertilizer		

[c] As superphosphate.
[d] As muriate of potash.
[e] The source of nitrogen for summer applications in 1930 contained 20 per cent N and 20 per cent lime (CaO).

EXPERIMENTAL PROCEDURE

The system of grazing and of taking grass samples has already been described in detail.[6] It was identical throughout except that in 1930 two of the plots in group A and the control plot (D) were divided in half transversely in order to facilitate uniformity and completeness of grazing.

Except for clacium and phosphorus, methods of chemical analysis were those of the Association of Official Agricultural Chemists.[7] Calcium was determined volumetrically by McCrudden's method and phosphorus colorimetrically by the method of Fiske and Subbarow.[8] Botanical analyses of the herbage were made in 1930. The results are given in Table 2. Soil analyses were made in the fall of 1928 on groups A and D only. The results of these analyses appear in Table 3. The soil is a silt loam of the Merrimac series, of level topography. Practically all the 74-acre tract has been cleared, drained, and brought under cultivation within a very few years. Previous to 1928 the tract had been included in a regular form rotation of corn, 2 years; hay, 2 years; and pasture, 2 or 3 years. The plots in group B were seeded for the first time in 1926.

The composite rainfall and temperature records for the three growing seasons are shown graphically in Figure 1 in comparison with the 40-year normal for Amherst, Mass. The weather was characterized by a normal month of May and a warm, wet June, the other months

[6] ARCHIBALD, J. G., and NELSON, P. R. Op. cit.
[7] ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. COMPILED BY THE COMMITTEE ON EDITING METHODS OF ANALYSIS. REVISED TO JULY 1, 1924. Ed. 2, 535 p., illus. Washington, D. C. 1925.
[8] FISKE, C. H., and SUBBAROW, Y. THE COLORIMETRIC DETERMINATION OF PHOSPHORUS. Jour. Biol. Chem. 66: 387–389. 1925.

having a normal temperature but showing a deficiency in rainfall, which became intensified as the season advanced.

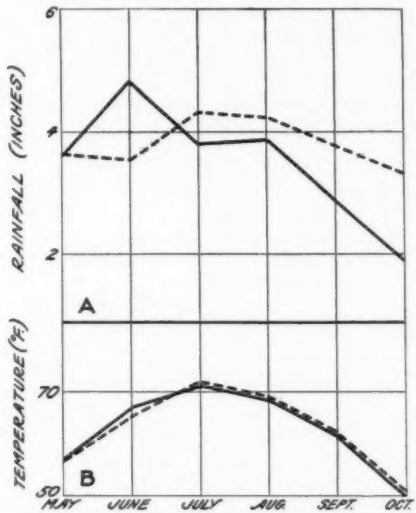

FIGURE 1.—Composite rainfall (A) and temperature (B) records for the growing seasons of 1928, 1929, and 1930. Dotted lines represent normals for Amherst, Mass.

TABLE 2.—*Botanical composition of the pasturage herbage on the several groups of plots in 1930*

Species	Group A		Group B		Group C		Group D	
	June	August	June	August	June	August	June	August
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Phleum pratense	21	19	23	25	21	20	7	7
Agrostis alba	20	24	39	33	32	37	45	50
Poa pratensis	46	42	27	31	30	25	25	15
Trifolium repens	4	4	1	Trace.	7	7	Trace.	3
Juncus spp.			3	3				
Other grasses [a]	5	7			5	5	8	5
Weeds	4	4	7	8	5	6	15	20

[a] Other grasses included varying amounts of *Dactylis glomerata, Anthoxanthum odoratum, Lolium italicum, Festuca elatior,* and *Poa trivialis.* Weeds included *Taraxacum* spp., *Ranunculus* spp., *Plantago* spp., *Potentilla* spp., *Alsine media, Chrysanthemum leucanthemum, Rumex acetosa, Achillea millefolium,* and *Carex* spp.

TABLE 3.—*Results of analyses of soil samples from plot groups A and D, taken in the fall of 1928*

Group	Fine soil (1 mm or less)	Organic and volatile matter	Total nitrogen	Total phosphorus	Total potassium	Total calcium	pH [a]	Lime requirement as CaO, by Jones's method
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*		*Pounds per acre*
A	98.4	6.63	0.175	0.170	1.17	0.136	5.86	7,136
D	98.0	6.08	.155	.148	1.10	.124	6.25	5,129

[a] pH determinations were made on samples taken in the spring of 1930. These values were also determined for Groups B and C; they were 5.47 and 5.90, respectively.

PRESENTATION OF RESULTS

EFFECT OF FERTILIZATION AND GRAZING ON THE COMPOSITION OF PASTURE GRASS

The chemical composition of the grass from all groups is shown in Table 4.[9] The results for Group B have been segregated from those for Group A because the plots in the former group grew a crop of hay each June and were not grazed until July, while those in the latter group were grazed throughout the season each year.

TABLE 4.—*Effect of a system of intensive fertilizing and grazing on the chemical composition (on a dry-matter basis) of pasture grass grown on experimental plots in 1928, 1929, and 1930*

[The values expressed in pounds per acre are true averages of the weights of the crop at the several times of sampling as total weights would not be comparable because of the varying number of samples in the different groups]

Plot group, number of samples, and treatment	Dry matter		Nitrogen		Crude fiber		Ether extract		Calcium		Phosphorus	
	Per cent	Pounds per acre	Per cent	Pounds per acre	Per cent	Pounds per acre	Per cent	Pounds per acre	Per cent	Pounds per acre	Per cent	Pounds per acre
A, average of 73 samples (complete fertilizer plus summer applications of nitrogen)	27.0	1,054	3.0	31.3	23.4	247	3.3	34.7	0.56	5.9	0.33	3.4
B, average of 39 samples (same fertilizer treatment as above but not grazed until July)	29.3	954	2.7	25.7	24.8	236	3.2	30.4	.60	5.7	.26	2.4
C (no nitrogen), average of 8 samples (superphosphate, muriate of potash)	34.2	886	2.4	21.6	23.8	211	3.3	29.5	.60	5.3	.27	2.4
D (control), average of 17 samples (unfertilized)	34.9	775	2.1	16.1	25.1	195	3.0	23.5	.54	4.2	.29	2.2

Considering the groups in reverse alphabetical order, a study of Table 4 shows that—

There was a gradual but not very uniform decrease in percentage of dry matter, accompanied by a corresponding but more uniform increase in amount of dry matter produced per acre. In other words, one effect of the fertilizer was to produce a considerably larger amount of more succulent grass, even on a dry-matter basis. In Group B, the effect of a hay crop in June with consequent postponement of grazing until July is seen in an increased percentage, and a decreased production, of dry matter. In contrasting C with A and also with D, the effect of a nitrogenous fertilizer in producing a succulent grass and a relatively large amount of dry matter is seen to be very marked.

There was a marked and regular increase in nitrogen content of the dry matter, and an even more marked though less uniform increase in nitrogen recovered per acre. This is the most striking and relatively much the greatest effect of the system, the amount of nitrogen in the samples from Group A being 42.9 per cent greater than in those from Group D, while the amount of nitrogen recovered was almost double (94.4 per cent greater). In Group B, no allowance being made for the hay crop produced in June, the percentage of nitrogen was

[9] It should be borne in mind in a study of Table 4 and of the discussion which follows that Groups A and D are the two between which fundamental comparisons are made. The results from Groups B and C are included merely for what additional information they may furnish.

28.6 per cent greater than in Group D, while nitrogen recovery was almost 60 per cent more. Group C, which received no nitrogen in 1929 and 1930, evidently had some in reserve in the roots from the application in 1928, being midway between B and D both in percentage and in amount recovered. A quality of herbage superior to that in Group D with somewhat more clover probably accounts for part of the difference.

The crude-fiber content decreased slightly but not uniformly, wherever fertilizer was applied. On the other hand, acre production of fiber increased considerably and quite uniformly. In this latter respect there is quite a close correlation between crude fiber and dry matter. The considerably higher fiber content in Group B than in either A or C is attributed to the presence of stubble from the June hay crop.

The content of ether extract was increased somewhat, and the quantity per acre was increased markedly by the treatment, but neither increase was uniform.

The calcium content of the grass was not materially affected by the fertilizer applications, Group A being only 0.02 per cent higher than Group D, which represents an increase of only 3.7 per cent. Acre recovery was of course considerably increased because of the much larger crop in Group A. The higher calcium content in Groups B and C probably is due to the presence of considerably more white clover (*Trifolium repens*) in the case of Group C, and to much greater amounts of red top (*Agrostis alba*) in both cases, than occurred in Group A. Recent unpublished work by the writers has revealed the fact that red top is somewhat higher in calcium than is either timothy (*Phleum pratense*) or Kentucky bluegrass (*Poa pratensis*). A liberal application of calcium nitrate [10] on the plots in Group B in 1928 is also considered to be a contributing factor.

The percentage of phosphorus was increased considerably in Group A as compared with that in Group D, and the acre recovery was over 50 per cent higher. The low value in Group B is rather difficult to interpret. It was consistently lower from year to year than that in Group D. The explanation offered in the published results for 1929 [11] is again advanced, to the effect that—

> these plots have been brought under cultivation comparatively recently and as a consequence may not have accumulated a phosphorus reserve comparable with that on the first six plots [Groups A and D], which had been fertilized quite liberally over a considerable period of years previous to the commencement of the grazing experiment.

It is also possible that the growing of a hay crop to comparative maturity may account for the disappearance of a considerable share of the store of available phosphorus on these plots. The low value in Group C can not be accounted for on any reasonable basis.

To summarize briefly, the most outstanding effect of the system has been to increase considerably the dry matter produced per acre and at the same time lower the dry-matter content of the fresh grass, and to increase greatly the nitrogen content of the dry matter. All other constituents determined, crude fiber excepted, were increased, although the effect on the calcium content of the grass was slight. Inasmuch as nutritive value of feeds is judged to a considerable extent

[10] For an explanation of the reason for using calcium nitrate, see the following publication: ARCHIBALD, J. G., and NELSON, P. R. Op. cit., p. 688, footnote to Table I.

[11] ARCHIBALD, J. G. Op. cit., p. 494.

by a minimum of fiber and a maximum of other proximate constituents, it may be assumed in the absence of actual feeding trials that the grass from Groups A, B, and C was superior as a feed to the grass from Group D, that from Group A being of especially high quality. The grass produced after a crop of hay was somewhat inferior in quality to that produced on plots that were continuously grazed. This is doubtless due in part to the presence of stubble from the hay crop. The effect of withholding nitrogen from Group C naturally was most apparent in the nitrogen content of the samples from that group.

SEASONAL VARIATIONS

As a basis for discussion and for the formulation of general conclusions on this phase of the work, the results from Groups A, C, and D have been combined in Table 5 and are shown graphically in Figure 2. This manner of handling the results was decided upon after it had been observed that in general the fertilizer applications had only a minor influence on the seasonal fluctuations in composition. This being the case, it was felt that by combining the groups the larger number of samples represented would contribute to more reliable final conclusions. Group B was not included because the growth of a hay crop on the plots in this group rendered the values from them not comparable with the others.

In Table 6 the detailed seasonal variations for all the groups are recorded separately, and those for Groups A and D are shown separately in Figure 4, for the sake of comparison with the composite graph in Figure 2 but not as a basis for general discussion. Groups B and C are not represented in Figure 4; the former for the reason given in the preceding paragraph, the latter because the number of samples (eight) when divided among six different months is insufficient to furnish reliable figures.

Figure 1, which gives the composite rainfall and temperature record for the three seasons should be studied in close conjunction with the detailed discussion which follows.

TABLE 1.—*Seasonal variations in chemical composition of pasture grass (dry-matter basis); average values for three seasons, 1928, 1929, and 1930*

[The values expressed in pounds per acre are true averages of the weights of the crop at the several times of sampling as total weights would not be comparable because of the varying number of samples in the different groups]

Month	No. of samples	Dry matter		Nitrogen		Crude fiber		Ether extract		Calcium		Phosphorus	
		Per cent	Pounds per acre	Per cent	Pounds per acre	Per cent	Pounds per acre	Per cent	Pounds per acre	Per cent	Pounds per acre	Per cent	Pounds per acre
May	20	26.5	608	3.6	21.7	16.9	103	3.6	22.1	0.51	3.1	0.36	2.2
June	13	21.7	1,324	2.8	37.2	24.8	328	3.3	43.9	.60	7.9	.36	4.7
July	13	27.8	1,170	2.5	29.6	26.5	310	2.9	34.2	.58	6.7	.32	3.8
August	19	30.6	1,238	2.6	32.1	26.3	325	3.1	38.1	.57	7.1	.31	3.8
September	17	28.7	911	2.8	25.6	24.4	222	3.3	30.5	.56	5.1	.31	2.8
October	16	34.8	769	2.6	19.9	21.9	168	3.3	25.7	.59	4.5	.28	2.1
Total	98												
Average [a]		27.7		2.8		24.0		3.2		.57		.32	

[a] The true average percentage composition of all the samples is recorded here merely as a matter of interest.

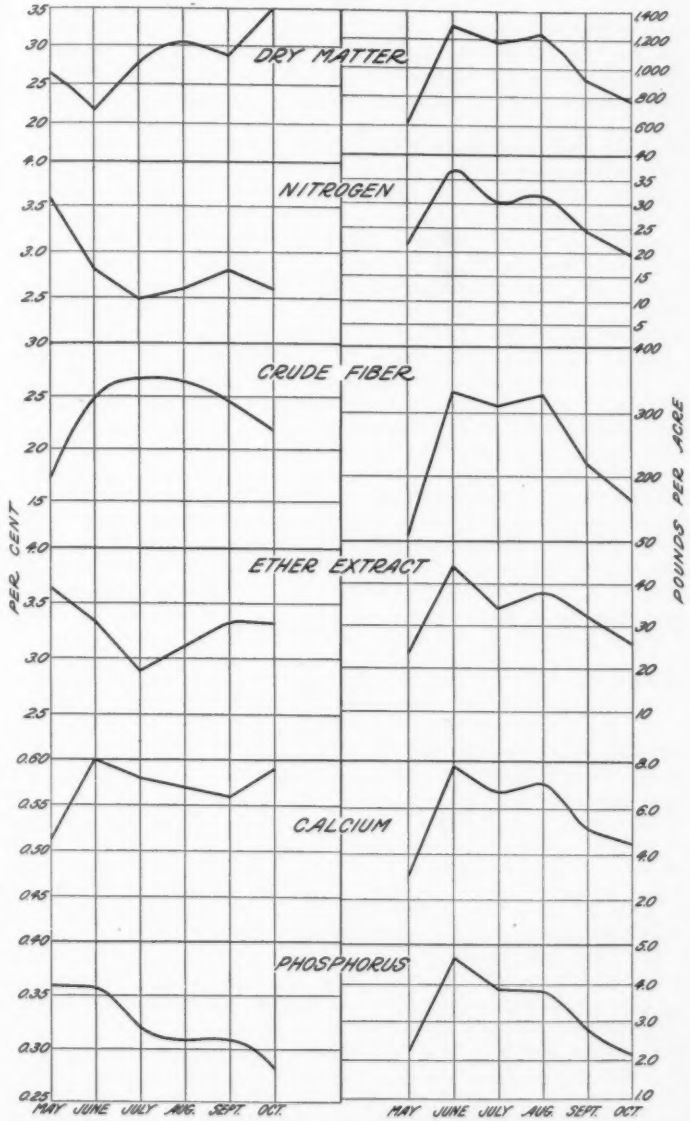

FIGURE 2.—Seasonal variations in the chemical composition of grass from plots fertilized and grazed intensively

Dry Matter

The curve for the dry-matter content of the grass (fig. 2), shows a general upward trend with the advance of the season. The decrease from May to June is considered to be due largely to the abundant moisture in June, although the presence of dead grass from the previous season's growth may have introduced an error that makes the values for May too high. In view, however, of the drop in nitrogen from May to June and the rise in crude fiber, the possibility of any considerable error in this connection seems remote. The drop in September is thought to be due partly to the late August and September rains and partly to the lower temperatures of autumn which result in less drying out of pastures. In accordance with this reasoning, it would seem that the values for October should be still lower. On the contrary, they are the highest for the season, which is attributed to gradual cessation of growth due to the action of frost.

Comparison of the dry-matter curve with the rainfall curve shows in general a close inverse relationship, although the changes in dry-matter content tend to lag somewhat behind the variations in rainfall.

The curve of dry-matter production has its high point in June with a slightly smaller peak in August. Despite a relatively very low dry-matter content in June, the ideal conditions for growth at that time, viz, abundant moisture and warmth, resulted in the highest dry-matter production of the season. In August the situation was the reverse of that in June—a relatively high dry-matter content offset a much lowered production of grass sufficiently to cause a second peak in the curve. The phenomenal rise in production of dry matter and all its constituents from May to June and the slump in July is clearly depicted in Figure 2.

Nitrogen

The general trend of the curve for nitrogen content of the dry matter is downward with the advance of the season. The marked drop from May to June is particularly worthy of note. It is considered to be due partly to the diminishing influence of heavy early spring applications of nitrogenous fertilizer, but this can not be the only cause as the curve for the control plot which had no fertilizer (fig. 4 (D)), also shows a decrease, although it is much smaller. It has been suggested that root reserves of nitrogen play a part here, being drawn upon extensively for the early spring growth. Also the much greater yield of grass in June than in May probably contributed to the lowered nitrogen content. The considerable rise in nitrogen from July to September is attributed to the influence of the summer applications of nitrogen, as this increase was not manifest in the samples from the control plot. The inverse relationship between nitrogen content and crude-fiber content, except at the end of the season, is very striking.

The curve for nitrogen recovery [12] is of the same general form as that for dry-matter production, as indeed are the production (or recovery) curves for all constitutents determined. The form is that of a grotesquely sprawled letter M. In this case the June peak is considerably above the secondary peak in August. The July slump is more marked here than it is in the dry-matter curve. The similarity to the rainfall

[12] The term "recovery" is used instead of "production" in those three cases in which the constituent under discussion is an element (nitrogen, calcium, or phosphorus).

curve (fig. 1) indicates a close relationship between amount of rainfall and nitrogen recovery in the grass.

Crude Fiber

The curve for the percentage of crude fiber in the dry matter is the most regular of all. Commencing at much the lowest value in May it rises rapidly to its peak in July, dropping off as regularly but somewhat more slowly as the season wanes. Part of this seasonal fluctuation probably is due to differences in stage of maturity of the samples, but since all samples were taken in the vegetative stage it would seem that there must be other factors of equal or greater importance in this connection. It seems reasonable to ascribe the fluctuations in fiber content largely to the interaction of two important growth factors, viz, moisture and sunshine. So long as the amount and intensity of sunshine is on the increase, which is the case until July, the process of fiber building proceeds rapidly. After June, the increase in fiber is further enhanced relatively by the drying up of other plant tissues which follows in the wake of diminishing moisture supply with the advance of the season. Midsummer or soon thereafter seems to be the peak of the combination or interaction of these two factors, hence the high fiber values for July and August. The decrease in fiber content with the advent of autumn may be due in part to the fact that the samples were less mature than those taken in midsummer, but is ascribed largely to rapid diminution in the amount of sunshine, especially so because the moisture supply remained scanty to the end of the season. This latter fact strengthens the assumption regarding the great influence of sunshine on fiber content for two reasons, (1) if sunshine were not an important factor, the fiber content should increase rather than decrease because of the drying-out effect of an increasing shortage of moisture; and (2) because of the dry autumn, conditions were not favorable for the production of a succulent new growth to which the decreased fiber content might otherwise be attributed.

The principal points of interest brought out by the curve for production of fiber are (1) the very large increase from May to June, which is relatively higher than for any other constituent, and especially high in contrast with nitrogen and ether extract, and (2) the secondary peak in August is of the same magnitude as that for June, while in the case of all other constituents it is somewhat smaller.

Ether Extract

There is a sharp decrease in the percentage of ether extract from May to July, followed by a somewhat less rapid increase until September. In general the curve resembles that for nitrogen content, and reasons similar to those given for nitrogen fluctuations can be assigned here. That these two constituents are quite closely associated in their ups and downs is further evidenced by the striking similarity of their production curves.

Calcium

There is some correlation, although it is by no means close, between calcium content and crude-fiber content. The same marked increase from May to June and the downward trend later is noted in both

cases, although it commences sooner in the case of calcium. It is known that one of the functions of calcium in the plant is as a constituent of the middle lamella of the cell wall, so that a reasonable correlation ought to be evident here. The rise in calcium content in October is probably traceable to the calcium present in summer applications of nitrogenous fertilizers that carried considerable calcium. A study of Figure 4, in which the fertilized and control plot values have been separated, confirms this.

Calcium recovery closely parallels dry-matter production, with a slightly greater peak in June.

Phosphorus

With phosphorus the trend is more definitely downward all through the season than with any other constituent determined. It does not drop so rapidly in the early part of the season as does either nitrogen or ether extract, but the increase later on, which characterizes these, is absent here.

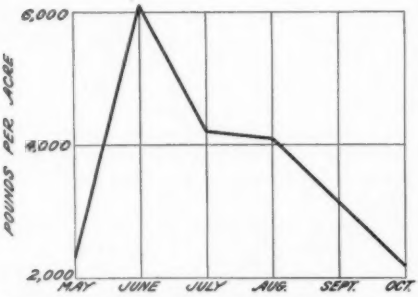

FIGURE 3.—Seasonal variation in production of fresh grass (composite for all plots)

The curve for recovery of phosphorus differs from all others in not having a secondary peak in August, although there is a break in its downward course at that point. Except for the much sharper rise from May to June it resembles the rainfall curve (fig. 1) very closely. The inference is that in dry seasons there may be a shortage of phosphorus in pastures. This is in agreement with the findings of other investigators.

Comparison of Seasonal Variations in Grass From Plots Receiving Different Fertilizer Treatments

Considering the seasonal differences in composition as a whole, it is apparent that from the standpoint of quality the grass on these plots was at its best in May, the content of nitrogen, phosphorus, and ether extract being at its maximum then, in association with a minimum of crude fiber and a dry-matter content somewhat above that for June. These facts confirm the old idea that the "early bite" is particularly good feed.

From the standpoint of quantity, the June growth naturally surpassed that for all other months, and except for the fact that it was somewhat more watery, it was comparable in quality with that grown in September, being superior to the September grass in mineral content. The slump in both quantity and quality in July was probably inevitable, but it is interesting to note the improvement in quality which commenced with the passing of midsummer and continued into September. Coupled with a definite increase in August in production of dry matter and of all the constituents except phosphorus, this

improvement indicates that August and September pastures have greater potentialities than are generally recognized. It should be noted, however, that this August increase was due not to an increase in the amount of green grass produced, but to a sharp slackening in

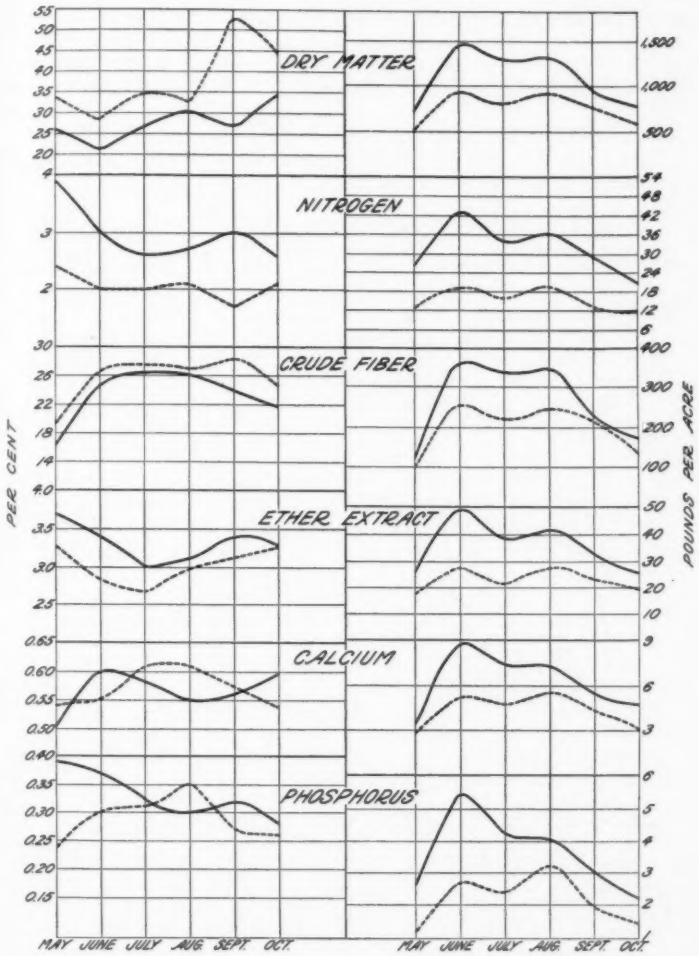

FIGURE 4.—Seasonal variations in the composition of grass from fertilized and from unfertilized plots, groups A and D only. Group A, solid line; Group D (control), broken line

the rate of decrease in production of green grass (fig. 3), accompanied by a relatively high dry-matter content.

Although in the nature of things uniform production and quality throughout the season are impossible of attainment, it is believed that some of the irregularities could be smoothed out considerably. If some of the June peak could be pushed over into the July depression

and if the relatively high quality of September grass could be attained a month earlier, one of the difficulties of pasture management would be minimized considerably. Less fertilizer in the spring and more in the summer would be a step in this direction, if summer labor conditions permitted such a change of plan. The problem is one for the agronomist and farm-management specialist.

TABLE 6.—*Seasonal variations in chemical composition (on a dry-matter basis) of pasture grass grown on plots fertilized with and without nitrogen, and on check plots, in 1928, 1929, and 1930*

Plot group, month, and treatment	Dry matter		Nitrogen		Crude fiber		Ether extract		Calcium		Phosphorous	
	Per cent	Pounds per acre	Per cent	Pounds per acre	Per cent	Pounds per acre	Per cent	Pounds per acre	Per cent	Pounds per acre	Per cent	Pounds per acre
A (Complete fertilizer plus summer applications of nitrogen):												
May	25.1	665	3.9	25.9	16.2	108	3.7	24.7	0.50	3.3	0.39	2.6
June	20.7	1,450	3.0	43.2	24.6	357	3.4	49.2	.60	8.7	.37	5.4
July	26.8	1,265	2.6	33.2	26.4	333	3.0	37.3	.58	7.3	.32	4.1
August	29.5	1,325	2.7	35.9	26.1	346	3.1	41.5	.55	7.3	.30	4.0
September	26.0	942	3.0	28.7	23.8	224	3.4	31.9	.56	5.3	.32	3.0
October	34.3	791	2.6	20.8	21.6	171	3.3	25.9	.60	4.7	.28	2.2
B (Same fertilizer as above but not grazed until July):												
May	23.6	711	3.8	27.3	19.0	135	3.6	25.3	.55	3.9	.34	2.4
June [a]												
July	26.8	1,149	2.6	29.5	27.1	311	3.0	34.6	.67	7.6	.27	3.1
August	32.5	1,049	2.5	26.1	26.4	277	3.0	31.6	.56	5.8	.25	2.6
September	30.2	880	2.5	21.6	24.8	154	3.3	29.2	.54	4.8	.24	2.1
October	38.0	863	2.4	20.4	23.2	200	3.3	28.5	.62	5.3	.21	1.8
C (No nitrogen, superphosphate, muriate of potash):												
May	32.6	416	2.7	11.2	18.6	78	3.6	14.8	.49	2.1	.30	1.3
June	22.0	1,323	2.9	38.6	21.7	287	3.7	48.4	.70	9.2	.32	4.3
July	34.4	976	2.2	21.9	26.7	261	3.0	29.0	.50	4.9	.33	3.2
August	47.6	1,352	2.2	30.2	26.4	357	2.7	35.8	.73	9.8	.22	3.0
September	45.0	867	2.1	17.9	25.2	219	3.3	28.8	.54	4.7	.24	2.1
October	32.0	871	2.7	23.6	21.9	191	4.1	35.9	.57	5.0	.25	2.2
D (Control) unfertilized:												
May	33.0	506	2.4	12.2	19.0	96	3.3	16.6	.54	2.8	.24	1.2
June	28.1	946	2.0	18.6	26.8	254	2.8	26.7	.55	5.2	.30	2.8
July	34.4	790	2.0	15.7	27.7	219	2.7	21.4	.61	4.8	.31	2.4
August	32.2	904	2.1	19.3	26.9	244	3.0	27.0	.61	5.5	.35	3.2
September	53.3	756	1.7	12.9	28.5	215	3.1	23.1	.57	4.3	.27	2.1
October	44.1	575	2.1	11.8	24.3	140	3.3	19.2	.54	3.1	.26	1.5

[a] Cut for hay.

Attention is directed briefly to Table 6 and Figure 4. In the latter a comparison is drawn between the values obtained on the fertilized plots and those obtained from the control plot. In general the curves are quite similar in trend. The high point of production in June was accentuated by the use of fertilizer, but the high point in August (phosphorus excepted) was of similar magnitude in both cases. The tendency for the curves to approach each other at each end of the season while being relatively well separated from June to September, is worthy of note.

It is interesting to observe the relatively great difference in both composition and production between the two groups in May as regards nitrogen, phosphorus, and ether extract.

The curves for content of calcium and phosphorus are an exception to the general similiarity in the fluctuations of the two groups. One

effect of the fertilizer seems to have been to speed up assimilation of calcium early in the season with the result that the highest value and the subsequent decrease came about a month earlier than they did on the check plot. The second rise in the curve for fertilized plots from August onward is attributed, as already stated, to the readily soluble calcium contained in summer applications of nitrogenous fertilizers carrying considerable calcium. It is thought that the curve for the check plot more nearly represents the normal seasonal changes in calcium content. It is in agreement with the curves for calcium given by Orr.[13]

A similar stimulating effect of the fertilizer on phosphorus assimilation is evident, the high point for phosphorus content coming in May where fertilizer was applied, but not until August on the control plot.

SUMMARY AND CONCLUSIONS

The results of analyses of 137 samples of grass taken during the growing seasons of 1928, 1929, and 1930 from nine 8-acre pasture plots which were fertilized and grazed intensively are reported, discussed, and interpreted. The fertilizer treatment varied slightly from season to season, but the general plan involved the application of a concentrated complete fertilizer in early spring, followed by several summer applications of a concentrated nitrogenous fertilizer. The plots were grazed in rotation and stocked with sufficient cattle to keep the grass quite short. Samples of the herbage on each plot were taken at the commencement of the pasture season in May, and at the conclusion of the rest periods of the plots, just before the cattle were turned in. Dry matter, total nitrogen, crude fiber, ether extract, calcium, and phosphorus were determined.

The most outstanding effects of the system were to increase considerably the dry matter produced per acre and at the same time to lower the dry-matter content of the fresh grass, and to increase greatly the nitrogen content of the dry matter. All other constituents, crude fiber excepted, were increased, although the effect on the calcium content of the grass was slight. The effect of nitrogen in producing a succulent grass and a relatively large amount of dry matter was very marked.

Inasmuch as the nutritive value of feeds is measured somewhat by a minimum of fiber and a maximum of other constituents, it may be assumed, in the absence of actual feeding trials, that the grass from the fertilized plots was superior as a feed to that from the control plot.

A study of the seasonal variations in 98 of the samples showed that the quality of the grass on these plots was at its best in May, and that in quantity of grass, June surpassed all the other months. Except in its mineral content, the September grass was comparable in quality with that grown in June.

The decrease in production in July while very evident was followed by just as evident an increase in August, some constituents showing nearly as high a production then as they did in June. This August increase was in dry matter, not in fresh grass.

Except in the case of the minerals (Ca and P), the assimilation of which it evidently stimulated, the fertilizer treatment had little effect

[13] Orr, J. B., with the assistance of SCHERBATOFF, H., MINERALS IN PASTURE & THEIR RELATION TO ANIMAL NUTRITION. P. 38–39. London. 1929.

on seasonal fluctuations in composition. It did, however, accentuate markedly the peak of production in June.

There is a close correlation between amount of rainfall and production or recovery of the more valuable constituents of the grass. This is especially true of the nitrogen, phosphorus, and ether extract.

Grass kept in the vegetative stage by grazing may be quite different in chemical composition in midsummer from what it was in the spring. The growth factors, notably the amount of rainfall and sunshine, exert their influence irrespective, to some extent at least, of the stage of growth of the plant.

The practical suggestion which is the obvious corollary to this study is that an attempt should be made to lessen these seasonal irregularities if and where possible. Less fertilizer in the spring and more in the summer is suggested as one remedy, if it be practicable.

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